

# STANDARD OPERATING PROCEDURE

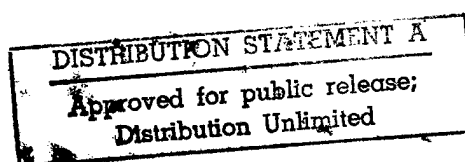
Red Blood Cells Collected in the CPDA-1 800 ml  
Primary PVC Plastic Bag Collection System and  
Stored for 3 to 6 Days Prior to Glycerolization in  
the Primary 800 ml Bag Using 40% W/V Glycerol and  
Storage at -80 C, Washed in the Haemonetics Blood  
Processor 115, and Stored at 4 C for up to 24 Hours  
Prior to Transfusion

Technical Report 97-08

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Development Command).

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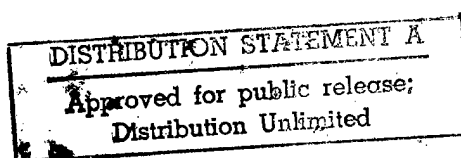


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**Reviewed and Approved****Date**

## INTRODUCTION

This is a Standard Operating Procedure (SOP) to glycerolize and deglycerolize human red cells in the 800 ml primary polyvinylchloride (PVC) plastic collection bag system. The glycerolized red cells are concentrated and the supernatant glycerol removed prior to freezing. The glycerolized red cells are washed in the Haemonetics Blood Processor 115.

Highlights of this revised SOP include a sterile docking procedure for glycerolizing the red blood cells, a change in centrifugation speeds, and new procedures for thawing the red blood cells prior to deglycerolization.

Freezing in the primary PVC collection bag reduces the potential for contamination, eliminates the need for a special freezing bag, reduces the volume of wash solution, permits biochemical modification of indated and outdated red cells, increases the storage capability of the -80 C mechanical freezer, and reduces the cost of freezing a unit of red cells by 25%.

The consumables and equipment described in this Standard Operating Procedure are FDA-approved and commercially available.

## COLLECTION AND PREPARATION OF BLOOD COMPONENTS

### I. INTRODUCTION

Established phlebotomy guidelines are to be followed. Be sure to adjust the balance or vacuum assist device so that only 450 ml of blood is collected into the 800 ml primary bag of the quadruple plastic bag collection system (Cutter #746-74; Fenwal #4R1243) (Figure 1).

The 800 ml primary collection bag contains 63 ml of citrate-phosphate-dextrose-adenine (CPDA-1) anticoagulant. After collection of 450 ml of blood in the primary collection bag, the unit is processed as follows:

#### FRESH WHOLE BLOOD

Within 8 hours of collection the primary bag is inverted and the bag is then folded back about 2 inches from the base and secured with tape. The folded bag is placed upright in a refrigerated centrifuge. The blood is centrifuged at 1615 X g for 4 minutes (no brake) to prepare a red cell concentrate with a hematocrit value of  $75 \pm 5$  V% (Table 1). All the platelet-rich plasma is removed to one of the integrally attached transfer packs and this transfer pack is detached from the primary bag. From this platelet-rich plasma two components may be prepared, each in a separate transfer pack: platelet concentrate, platelet-poor fresh frozen plasma, cryoprecipitate, or cryoprecipitate-poor fresh frozen plasma. The red cells are stored in the 800 ml primary bag at 4 C for 3 to 6 days (inclusive) and then frozen (Figure 1). Ideally, a non-anticoagulated blood sample for serum should be collected from the venipuncture site after the unit of whole blood is collected. The serum is transferred to three 2 ml polyethylene cryogenic vials for freezing and future testing. If sera is not available, prepare three 2 ml polyethylene cryogenic vials of plasma within 24 hours of collection for freezing for future testing of the plasma.

#### STORED WHOLE BLOOD OR RED CELL CONCENTRATES WITH UNKNOWN HEMATOCRIT VALUES

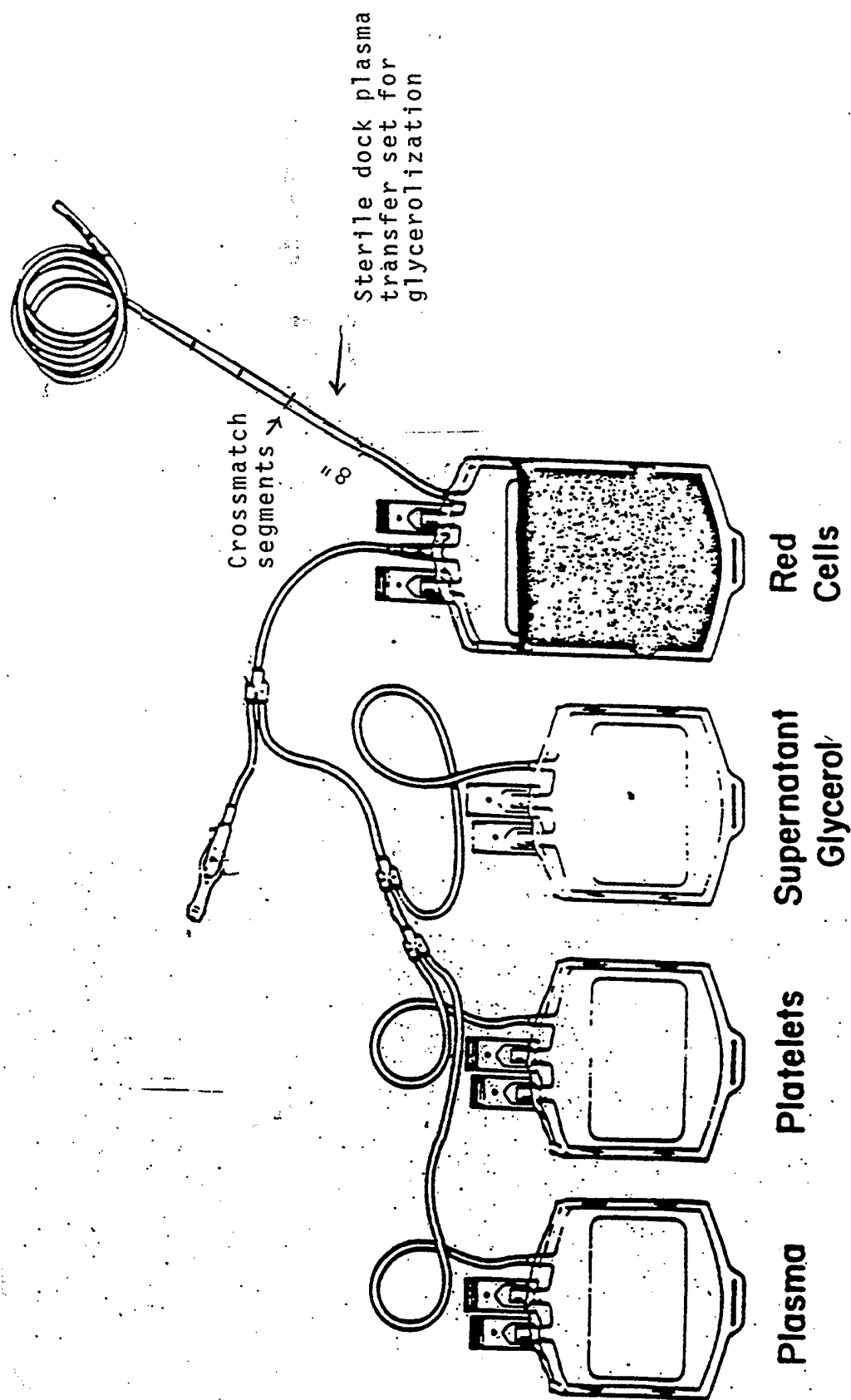
If the unit has been stored as whole blood at 4 C, or if the hematocrit of the red cell concentrate is unknown, the primary bag is inverted and the bag is folded back about 2 inches from the base and secured with tape. The folded bag is placed upright in a 22 C refrigerated centrifuge and centrifuged at 1615 X g for 4 minutes (no brake) to remove the plasma, and a red blood cell concentrate with a hematocrit of  $75 \pm 5$  V% is prepared. All the visible plasma is removed. Ideally, a non-anticoagulated blood sample for serum should be collected from the venipuncture site after

the unit of whole blood is collected. The serum is transferred to three 2 ml polyethylene cryogenic vials for freezing and future testing. If sera is not available, prepare three 2 ml polyethylene cryogenic vials of plasma within 24 hours of collection for freezing for future testing of the plasma.

**NOTE:** A small rubber band (size 8) or hand sealer clip is placed on the tubing connecting the primary bag to the transfer pack.

## **II. CONSUMABLES**

1. 800 ml CPDA-1 quadruple blood pack (Cutter #746-74; Fenwal #4R1243)
2. Rubber bands (size 8; 7/8 X 1/16 X 1/32) or hand sealer clips (Fenwal #4R4418)
3. 2 ml Polyethylene cryogenic vials (Corning 25702 or Fisher 03-374-6)



## 800 ml PRIMARY PVC PLASTIC BAG COLLECTION SYSTEM

FIGURE 1

TABLE 1

## SPEED AND LENGTH OF TIME FOR PROCESSING BLOOD COMPONENTS

1. Fresh whole blood spun at 1615 x g for 4 minutes in a 22 C refrigerated centrifuge (no brake) to prepare platelet-rich-plasma (PRP) and a red blood cell concentrate with a hematocrit value of  $75 \pm 5$  V%.
2. Liquid preserved whole blood or red cell concentrates with unknown hematocrit values, spun at 1615 x g for 4 minutes in a 22 C refrigerated centrifuge (no brake) to a red blood cell concentrate with a hematocrit value of  $75 \pm 5$  V%.
3. Non-rejuvenated glycerolized red cells spun at 1248 X g for 10 minutes in a 22 C refrigerated centrifuge (no brake) to prepare a glycerolized red blood cell concentrate with a hematocrit value of  $60 \pm 5$  V%.
4. Deglycerolized red cells resuspended in NaCl-glucose solution spun at 2982 x g for 4 minutes in a 22 C refrigerated centrifuge (no brake) to remove supernatant hemolysis and prepare a red cell concentrate prior to transfusion with a hematocrit value of 85 V%.

$$\text{Where; } RCF = (28.38) (R) \left( \frac{RPM}{1000} \right)^2$$

RCF = RELATIVE CENTRIFUGAL FORCE (X g)

R = RADIUS IN INCHES

RPM = REVOLUTIONS PER MINUTE

RADIUSROTOR

R =	<u>9.09</u> Inches for the <u>HG-4L OR H4000</u> 4-Bucket rotor used in the Sorvall RC-3B or RC-3C centrifuges.
R =	<u>10.25</u> inches for the <u>H6000A</u> 6-bucket rotor used in the Sorvall RC-3B or the RC-3C centrifuges.
R =	<u>8.90</u> inches for the <u>JS5.2</u> 4-bucket rotor used in the Beckman J6-B centrifuge.
R =	<u>10.05</u> inches for the <u>JS4.2</u> 6-bucket rotor used in the Beckman J6-B centrifuge.

## GLYCEROLIZATION

### I. INTRODUCTION

A method is described for the preparation and storage of red blood cells, frozen, in the 800 ml primary polyvinylchloride (PVC) plastic collection bag. Four hundred fifty (450) ml of blood is collected in the 800 ml primary PVC plastic collection bag containing 63 ml of CPDA1. The unit is stored at 4 C for up to 6 days (inclusive) before glycerolization and freezing. Just prior to glycerolization, the red cells are spun at 1615 X g for 4 minutes in a 22 C refrigerated centrifuge to prepare a red cell concentrate with a hematocrit value of  $75 \pm 5$  V%. The special adaptor port on the tubing connecting the primary 800 ml PVC plastic bag to the three transfer packs is no longer used. Glycerol solution is now added through the donor collection line using a sterile dock procedure. Three aliquots of glycerol solution are introduced with short equilibration periods between each addition. The red cells are then concentrated to a hematocrit value of  $60 \pm 5$  V% by centrifugation at 1248 X g for 10 minutes, and the supernatant glycerol is transferred into the empty integrally attached transfer pack and discarded. The glycerolized packed red cells are then frozen. The procedure uses a high concentration of glycerol (40% W/V) and mechanical refrigeration at -80 C for freezing and storage of the product. Both the volume of blood collected (450 ml) and the hematocrit of the red cell concentrate ( $75 \pm 5$  V%) prior to glycerolization ensure that the 800 ml primary PVC plastic bag is adequate for proper mixing of the red cells and glycerol solution.

### II. STARTING COMPONENTS

Red cell concentrates (hematocrit value of  $75 \pm 5$  V%) in the 800 ml primary PVC plastic collection bag with an integrally attached empty transfer pack are stored at 4 C for 3 to 6 days (inclusive) prior to glycerolization.

### III. MATERIALS

#### CONSUMABLES

##### 1. Glycerol:

- A. Glycerolyte 57 (6.2 M glycerol, 500 ml bottle). Each 100 ml contains 57 g glycerin, 1.6 g sodium lactate, and 30 mg potassium chloride, buffered with 51.7 mg monobasic sodium phosphate (monohydrate) and 124.2 mg dibasic sodium phosphate (dried), pH 6.8 (Fenwal #4A7833)



B. 6.2 M Glycerolizing Solution (500 ml bottle). Each 100 ml contains 57.1 g glycerin, 1.6 g sodium lactate, and 0.03 g potassium chloride, buffered with 43 mg monobasic sodium phosphate and 220 mg dibasic sodium phosphate, pH 7.0 (Cytosol PN-5500)

2. Plasma transfer set with a coupler and needle adapter (Fenwal #4C2240)
3. Sterile docking waters (Terumo 3NCC987)
4. Sterile filtered airway needle (B-D 5200)
5. Labels for the primary collection bag and for the cardboard storage box
6. Heat-sealable plastic bags (3), 8" X 12" (Kapak/Scotchpak 404)
7. Corrugated cardboard storage box. (Dimensions: 7" X 5.25" X 2" outside)
8. Alcohol swab (70%) (B-D 6894)

#### IV. TEMPERATURE REQUIREMENTS

At the time of glycerolization, the red cells, glycerol solution and room temperature should be within a temperature range of 20 C (68 F) to 30 C (86 F). The temperature of a bottle of glycerol located in the storage area should be monitored by inserting a calibrated thermometer into the full bottle of glycerol. If the glycerol is below 20 C, the glycerol can be warmed to a temperature of 20-26 C by incubation at 37 C for the appropriate time to achieve the desired temperature. The red cells can be warmed by one of the following manipulations:

- A. Remove the liquid red cell concentrates from the 4 C refrigerator and place them in sealed, double plastic bags for protection against wetting. Immerse the double-bagged units in a 37 C water bath for 20 minutes. See the videotape for instructions.
- B. Remove the liquid red cell concentrates from the 4 C refrigerator and place each unit in a Thermogenesis plasma thawer pouch for 4 minutes at 40 C.

- C. Remove the liquid red cell concentrate from the 4 C refrigerator and store at room temperature for a period of about 2 hours.

The water bath and plasma thawer procedures are outlined below.

#### **WATER BATH (BLUE-M, MODEL MW1140A)**

**NOTE:** Each plastic overwrap bag must be flattened to remove all the air prior to sealing. If this is not done properly, the units will float on the surface of the water bath during incubation, and the desired temperature will not be achieved.

1. The primary collection bag containing the red cell concentrate and integrally attached transfer pack are placed in a plastic bag, and the bag is heat sealed.
2. The sealed plastic bag is placed inside a second plastic bag; this bag also is heat-sealed.
3. Turn on the power switch of the water bath located at the end of the water bath. Allow the water to warm to 37 C (approximately 1 hour). Switch on the circulating pump in the water bath a few minutes prior to use to ensure a uniform temperature of 37 C throughout the bath. Temperature is measured with a thermometer that has been verified against a National Institute of Standards and Technology (NIST) certified thermometer.
4. Place the overwrapped unit(s) in the water bath, placing lead weights on the bag to keep it submerged.
5. Incubate the unit in the 37 C water bath for 20 minutes; at the end of 20 minutes the temperature of the red cells should be 20 C-30 C. Measure the surface temperature of the unit with an infrared scanner or NIST certified thermometer.

6. Remove the bag from the water bath; wrap the unit loosely in a clean, dry disposable towel, dry the surface of the overwrap, and remove the plastic overwraps from the primary bag, assuring that the inner bag and unit are not contaminated with any water from the water bath.
7. The red cells are now ready for glycerolization.

#### **PLASMA THAWER (THERMOGENESIS, MODEL MT204)**

**NOTE: Plastic overwrap bags are not required when the plasma thawer is used.**

1. Turn on the power to the plasma thawer and allow the system to warm to 40 C.
2. Place a unit in each pouch of the plasma thawer. Allow the integrally attached transfer pack to hang over the side of the plasma thawer.
3. Set the timer for 4 minutes. At the end of 4 minutes the temperature of the red cells should be 20-30 C. Remove the unit from the pouch and measure the surface temperature of the unit with an infrared scanner or NIST certified thermometer.
4. The red cells are now ready for glycerolization.

#### **V. GLYCEROLIZATION**

1. Remove the numbered crossmatch segments from the donor collection line and leave at least 8 inches of tubing for sterile docking of a plasma transfer set to glycerolize the red cells.
2. Weigh and record the weight of the unit. To obtain the gross weight of the unit, weigh only the 800 ml primary bag containing the red cell concentrate. To obtain the net weight of the red cells, subtract 42 g (weight of empty 800 ml primary bag) from the gross weight.
3. Remove a plasma transfer set (Fenwal 4C2240) from its box, slide the roller clamp to the coupler end of the plasma transfer set, and close the roller clamp.
4. Sterilely dock the plasma transfer set (Fenwal 4C2240) to the donor collection line (Figure 1). Squeeze weld.

**NOTE:** The needle adapter end of the plasma transfer set is discarded when the sterile docking procedure is performed.

5. Place the 800 ml primary collection bag containing the red cells on the shaker platform; allow the attached transfer pack to hang over the shaker platform.
6. Remove the metal pull tab from the top of the glycerol bottle, swab the rubber stopper with an alcohol swab (70%), and then aseptically insert the coupler end of the plasma transfer set (Fenwal 4C2240) into the outlet port of the glycerol bottle stopper.
7. Insert a filtered airway needle into the vent port of the glycerol bottle stopper. As the bottle vents, invert the glycerol bottle and install it on the support stand hook provided on the shaker so that the rubber stopper on the bottle of glycerol is held 18 inches (45 cm) above the level of the primary bag on the shaker.
8. Using Table 2 and the previously recorded gross or net weight, determine the volume of glycerol solution to be added to the red cells during each of the three glycerol addition steps. Using the factory graduations as a guide, mark the volume of glycerol to be added for each of the three steps.
9. Switch the modified Eberbach shaker on low speed (180 oscillations/minute). Check to be sure the rubber band or hand sealer clip is in place on the line between the primary bag and the empty transfer pack to prevent the glycerol from flowing into the transfer pack.
10. Open the roller clamp of the plasma transfer set (Fenwal 4C2240) and add the first volume of glycerol from the solution bottle directly into the primary bag containing the red cells.
11. Close the roller clamp, turn off the shaker and equilibrate the red cells for 5 minutes.
12. Switch the modified Eberbach shaker on low speed.
13. Open the roller clamp and add the second volume of glycerol from the solution bottle directly into the primary bag containing the red cells.

14. Close the roller clamp, turn off the shaker and equilibrate the red cells for 2 minutes.
15. Remove unit from shaker.
16. Open the roller clamp and using continuous, vigorous manual agitation, allow the third volume of glycerol (final volume) to enter directly into the primary bag.

**NOTE:** During the addition of the third volume of glycerol, only 2 units should be processed at a time to ensure proper vigorous manual mixing, one unit held in each hand.

17. Close the roller clamp and heat seal the tubing between the empty bottle of glycerol and donor collection line. Leave approx. 7 inches of tubing on the donor collection line.
18. Discard the empty glycerol bottle and the plasma transfer set tubing.
19. Spin the glycerolized red cells at 1248 X g in a 22 C refrigerated centrifuge (Sorvall or Beckman) for 10 minutes (Table 1).

**NOTE:** The brake on the centrifuge should be set at zero. This brake setting will minimize red cell mixing which occurs as the rotor slows down from maximum to zero.

20. Carefully remove the unit from the centrifuge and place it on a plasma extractor.
21. Remove the rubber band or hand sealer clip from the tubing between the collection bag and transfer pack.
22. Express all visible supernatant glycerol from the primary bag into the transfer pack to achieve a hematocrit of  $60 \pm 5$  V%. When red cells appear in the cannula, clamp the integral tubing with a hemostat. Remove the primary bag from the plasma extractor and resuspend and mix the glycerolized red cells thoroughly by manual agitation. The glycerolized red cell concentrate must be resuspended completely before freezing to prevent hemolysis.

23. Place the unit back onto the plasma extractor. Unclamp the hemostat and allow the mixed glycerolized red cells to fill the integral tubing (future crossmatching samples). Just before the red cells enter the transfer pack containing the supernatant glycerol, clamp the integral tubing, heat seal the transfer pack as close to the base of the transfer pack as possible, and discard. Also, heat seal and discard the adaptor port at this time. Heat seal and detach crossmatch segments, and freeze alongside the cryogenic vials in the cardboard freezing box. Leave 6 inches of tubing attached to the primary bag.
24. Affix the following labels to the primary bag:
  - A. Blood product overlay label to indicate that the product has been processed into "Red Blood Cells, Frozen" (Figure 2).
  - B. Freezing facility label (original facility label and manufacturer's name and bag lot number must be readable) (Figure 2).
  - C. ABO, Rh confirmation label (Figure 2).
  - D. Infectious disease testing label (affixed to the back-side of the primary bag) (Figure 3).
25. Mark the label with the expiration date of the blood product, which is currently 10 years from the day of collection (Figure 2). Weigh the unit just prior to freezing and record the gross weight of the glycerolized red cells.
26. Fold over the top portion of the primary bag (approx. 2 inches) and then place the unit into a plastic bag overwrap (8" X 12") and seal across the top using an impulse sealer so that there is as little trapped air as possible. The plastic bag will not break during freezing and the sealer will provide an air-tight and leakproof seal to ensure protection of the unit at the time of thawing. Make sure that the ports and tubing segments are folded beneath the unit so that they are protected from breakage when frozen (Figure 3).
27. Place at least one polyethylene cryogenic vial of plasma or sera into the cardboard box (Figure 4). The remaining two vials are frozen separately at -80 C.

28. Place the plastic bag containing the glycerolized red cells into the cardboard box and close the box (Figure 4). Affix a product label, ABO/Rh label (the expiration date must also appear on the label--currently 10 years from the date of collection), collection facility ID label, unit number label, freezing facility ID label, and label indicating infectious disease marker testing performed on the unit, on the outside of the box (Figure 5). Place the cardboard box in a -80 C freezer for freezing and storage. Each unit should be frozen at the bottom of the -80 C freezer during the initial 24-hour period to ensure proper freezing. To avoid improper freezing, the units should not be stacked on each other. After the initial 24-hour period of freezing at the bottom of the -80 C freezer, the frozen units can be stacked and stored in other -80 C freezers.

**NOTE:** No more than 4 hours should be allowed to lapse between the time the red cells are removed from the 4 C refrigerator and the time they are placed in the -80 C freezer. The final concentration of glycerol is approximately 40% W/V and the hematocrit of the glycerolized unit is approximately  $60 \pm 5$  V%.

DO NOT USE WITHOUT FURTHER PROCESSING.  
Deglycerolize after thawing. Failure to  
deglycerolize before administration  
could cause serious reaction.

Collect: **CMV  
NEGATIVE**  
258

4798111

EXPIRES **20 APR 1995**

FORM #5530

**RED BLOOD CELLS**  
FROZEN 06200

From 450 mL  
CPDA-1 Whole Blood.  
Store at -65° C. or colder. FORM #6025CM

See circular of information for  
indications, contraindications,  
cautions and methods of infusion.

**VOLUNTEER DONOR**

This product may transmit infectious agents.  
Caution: Federal law prohibits dispensing without  
a prescription.

**PROPERLY IDENTIFY INTENDED RECIPIENT**

**Rh NEGATIVE**

DEPARTMENT OF THE NAVY  
DIRECTOR, NAVAL MEDICINE  
Washington, DC 20350  
U.S. License #635  
Collected and Processed by  
NNMC Bethesda, MD 20889-5000

Registration #1176560  
FORM #1090-A

Baxter Healthcare Corporation  
Fenwal Division  
Deerfield, IL 60015 USA  
7-17-3-108 5JF304  
**PL 146**® Plastic

CODE 4R1245M  
LOT M94113068

DEPARTMENT OF THE NAVY  
Washington, DC 20372-5300  
Frozen by:  
Naval Blood Research Lab  
615 Albany Street  
Boston, MA 02118  
U. S. License No. 635

**ABO GROUP CONFIRMED**  
**Rh OF NEGATIVE UNITS CONFIRMED**  
16 MAR 95 FN

DATE/TECH

Registration #1283786



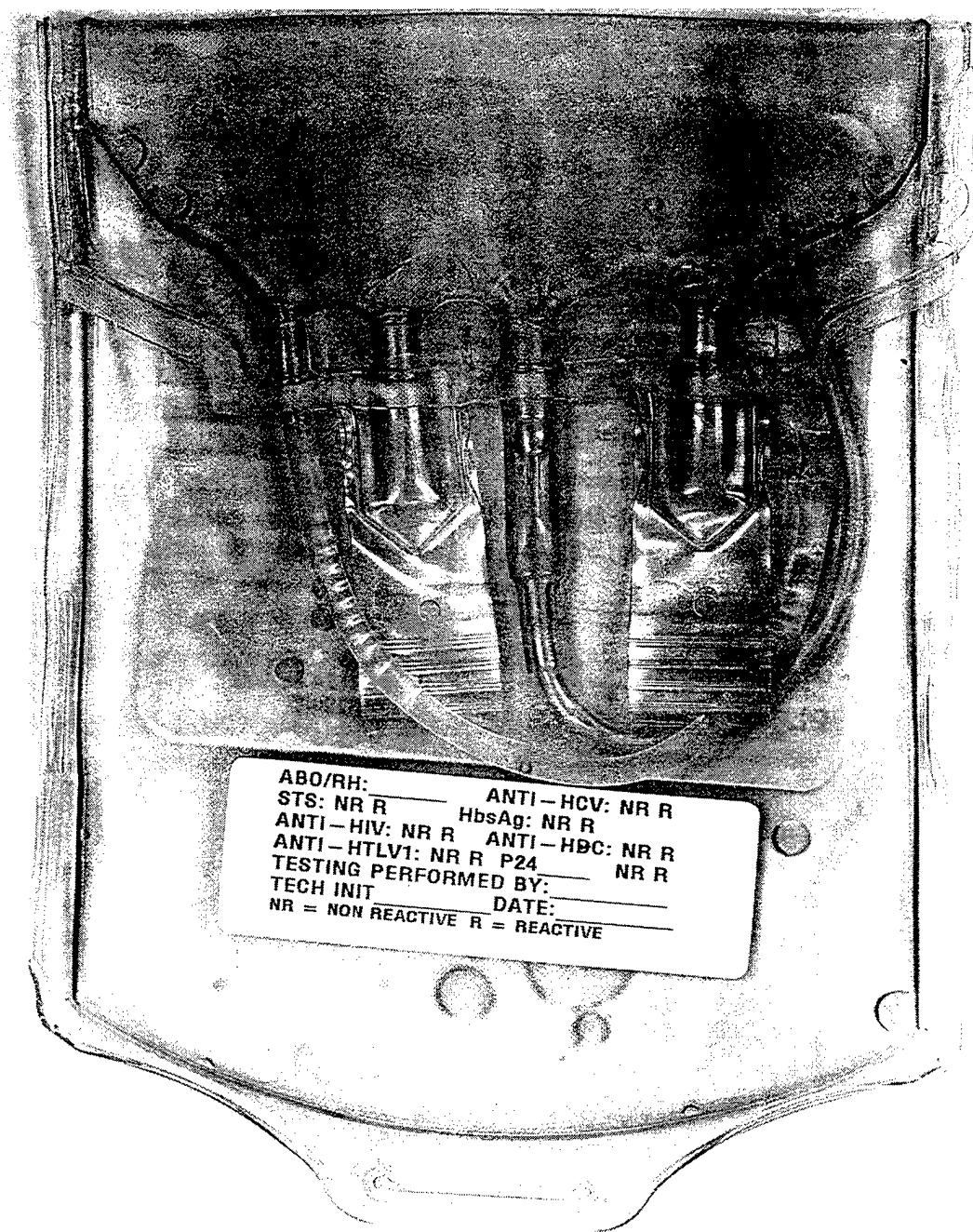
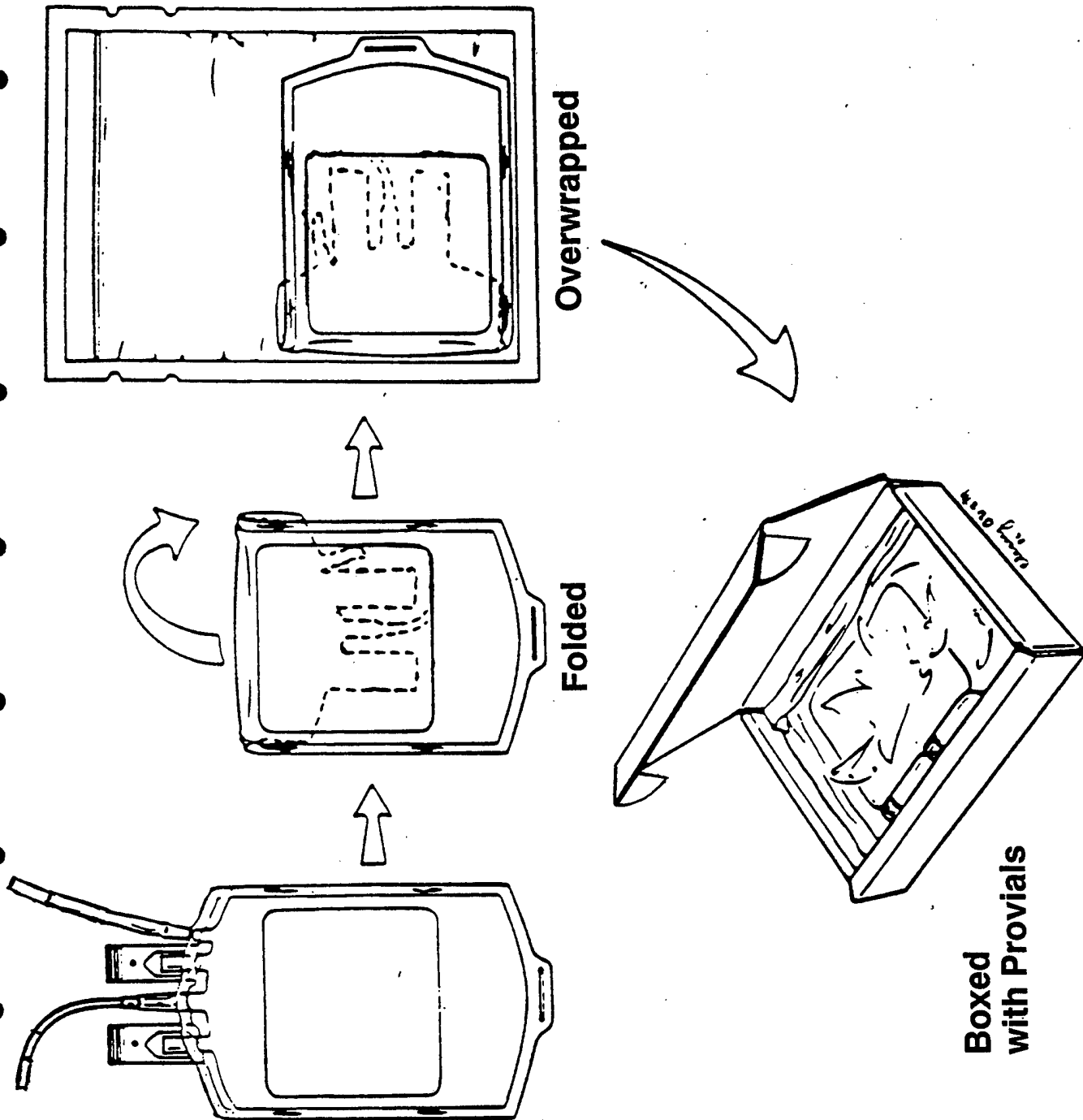


FIGURE 3



**FIGURE 4**

**RED BLOOD CELLS**  
**FROZEN** 06200  
 From 450 mL  
 CPDA-1 Whole Blood.  
 Store at -65° C. or colder.  
 FORM #603CM

**EXPIRES**  
**16 JAN 05**  
**0**  
**Rh POSITIVE**

4798111  
 DEPARTMENT OF THE NAVY  
 DIRECTOR, NAVAL MEDICINE  
 Washington, DC 20330  
 U.S. License #933  
 Donated and Procured by  
 Naval Medical Center  
 4000 Leno  
 Registration #1176560  
 FORM #1090-A

ABO/RH: \_\_\_\_\_  
 STS: NR R  
 ANTI-HIV: NR R  
 ANTI-HLV1: NR R  
 TESTING PERFORMED BY: \_\_\_\_\_  
 DATE: \_\_\_\_\_  
 NR = NON REACTIVE R = REACTIVE

DEPARTMENT OF THE NAVY  
 Washington, DC 20372-5300  
 Frozen by:  
 Naval Blood Research Lab  
 616 Albany Street  
 Boston, MA 02118  
 U.S. License No. 635  
 Registration #1293786

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 REF  
 REF  
 REF  
 AHE

FIGURE 5

TABLE 2

**METHOD OF ADDITION OF 6.2 M GLYCEROL TO NON-REJUVENATED AND  
REJUVENATED RED BLOOD CELLS**

GROSS WEIGHT OF UNIT (GRAMS) *	NET WEIGHT OF UNIT (GRAMS)	INITIAL ADDITION OF GLYCEROL (ML)	SECOND ADDITION OF GLYCEROL (ML)	THIRD ADDITION OF GLYCEROL (ML)	TOTAL GLYCEROL ADDED (ML)
193-242	151-200	50	50	250	350
243-282	201-240	50	50	350	450
283-422	241-380	50	50	400	500

\*Weight of the empty 800 ml primary plastic bag is 42 grams (average).

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REVISED 7/97

## GLYCEROLIZATION WORKSHEET

UNIT # \_\_\_\_\_ COLLECTION FACILITY \_\_\_\_\_ TYPE AND RH \_\_\_\_\_

DATE COLLECTED \_\_\_\_\_ IN VIVO \_\_\_\_\_ IN VITRO \_\_\_\_\_ WHY? \_\_\_\_\_

NBRL ACCESSION # \_\_\_\_\_ DATE FROZEN \_\_\_\_\_ FROZEN BY \_\_\_\_\_

## UNIT DESCRIPTION:

ANTICOAGULANT: CPD \_\_\_\_\_  
CP2D \_\_\_\_\_  
CPDA-1 \_\_\_\_\_

HOMOLOGOUS \_\_\_\_\_  
AUTOLOGOUS \_\_\_\_\_ NAME \_\_\_\_\_  
RARE \_\_\_\_\_  
HFD \_\_\_\_\_  
SPECIAL STUDY \_\_\_\_\_ IDENTIFY \_\_\_\_\_

INFECTIOUS MARKER TEST RESULTS      ARC \_\_\_\_\_ UMASS \_\_\_\_\_ OTHER \_\_\_\_\_  
NON REACTIVE (NEG)      REACTIVE (POS)

## SYPHILIS

HEP. B SURFACE ANTIGEN \_\_\_\_\_

ANTIBODY TO HTLV-1 \_\_\_\_\_

ANTIBODY TO HIV 1/2 \_\_\_\_\_

ANTIBODY TO HEP. CORE ANTIGEN \_\_\_\_\_

ALANINE AMINOTRANSFERASE (ALT) \_\_\_\_\_

ANTIBODY TO HEPATITIS C (HBC) \_\_\_\_\_

P24 ANTIGEN \_\_\_\_\_

PREPARATION OF RBC CONCENTRATES (22 C CENTRIFUGE WITH BRAKE OFF  
(SETTING AT ZERO)):

WITHIN 8 HOURS OF COLLECTION: 4 MINUTES AT 1615 X G \_\_\_\_\_ (75 V%)  
AFTER STORAGE AT 4 C: 4 MINUTES AT 1615 X G \_\_\_\_\_ (75 V%)  
(WHOLE BLOOD OR RBC CONCENTRATE WITH UNKNOWN HEMATOCRIT)

STORAGE AT 4 C: WHOLE BLOOD \_\_\_\_\_ DAYS      RBC CONCENTRATE \_\_\_\_\_ DAYS

RBC CONCENTRATE IN ADSOL, OPTISOL OR NUTRICEL \_\_\_\_\_ DAYS

## REJUVENATION (USING REJUVESOL)

INCUBATION TIME (37 C) \_\_\_\_\_ MIN TEMP. \_\_\_\_\_ C

## GLYCEROLIZATION:

SURFACE RBC TEMP \_\_\_\_\_ C      GLYCEROL TEMP \_\_\_\_\_ C      ROOM TEMP. \_\_\_\_\_ C

TIME BEGIN \_\_\_\_\_ (RBC PLACED IN WATER BATH, PLASMA THAWER OR ROOM TEMP)

TIME END \_\_\_\_\_ (RBC PLACED IN -80 C FREEZER)

TOTAL TIME AT ROOM TEMP. \_\_\_\_\_ (MUST BE LESS THAN 4 HOURS)

## METHOD OF WARMING 4 C NONREJUVENATED RBC:

2 HOURS AT ROOM TEMP \_\_\_\_\_ 20 MINUTES IN 37 C WATER BATH \_\_\_\_\_  
\_\_\_\_\_ MINUTES IN 40 C THERMOGENESIS

(OVER)

## GLYCEROLIZATION (CONTINUED):

UNIT # \_\_\_\_\_ NBRL ACCESSION # \_\_\_\_\_ DATE \_\_\_\_\_

GROSS RBC WEIGHT PRIOR TO ADDITION OF GLYCEROL \_\_\_\_\_ G

WEIGHT OF EMPTY PLASTIC BAG (INCL. TRANSFER PACK AND TUBING):  
800 ML: 72 G      1000 ML: 80 GWEIGHT OF EMPTY PLASTIC BAG ALONE:  
800 ML: 42 G      1000 ML: 44 G

NET WEIGHT OF RBC PRIOR TO ADDITION OF GLYCEROL \_\_\_\_\_ G

VOLUME OF 6.2 M GLYCEROL ADDED \_\_\_\_\_ ML

CENTRIFUGATION AT 22 C: BRAKE SET AT ZERO - 1248 X G FOR 10 MINUTES  
SORVAL RC3B \_\_\_\_\_ BECKMAN J6-B \_\_\_\_\_ SERIAL # \_\_\_\_\_

FINAL GLYCEROLIZED RBC CONCENTRATE:

GROSS WT \_\_\_\_\_ G - BAG WT 42 G - RBC WT \_\_\_\_\_ G  
BAG WT 44 GRECORD GLYCEROLIZATION END TIME ABOVE

## SUPPLIES:

	MANUFACTURER	LOT #	CAT. #	EXP. DATE
REJUVESOL	CYTOSOL	_____	PN-7012	_____
GLYCEROL	_____	_____	_____	_____
FREEZE BAG:	_____	_____	_____	_____
FENWAL 4R1243-	_____	_____	_____	_____
800 ml CPDA1				
CUTTER 746-74-				
800 ml CPDA1				
FENWAL 4R2986				
- 1000 ml				
PLASMA TRANSFER SET	FENWAL	_____	4C2240	_____
FOR GLYCEROLIZATION				
Y-TYPE TRANSFER SET	FENWAL	_____	4C1921	_____
FOR REJUVENATION				

## PROVIALS:

FROZEN AT -80 C WITH UNIT	YES _____	NO _____	# PROVIALS _____
FROZEN AT -80 C AT NBRL	YES _____	NO _____	# PROVIALS _____
FROZEN AT COLLECTION FACILITY	YES _____	NO _____	# PROVIALS _____

## INVENTORY CONTROL:

GLYCEROLIZATION LOG: YES \_\_\_\_\_ NO \_\_\_\_\_

COMPUTER INVENTORY LOG: YES \_\_\_\_\_ NO \_\_\_\_\_

COMMENTS: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

## THAWING

### I. INTRODUCTION

A unit of glycerolized frozen red cells can be thawed using one of two methods: a) rapid immersion into a heated water bath maintained at 42 C for approximately 45 minutes; or b) by placement into one of the pouches of a plasma thawer maintained at 40 C for 30-35 minutes. Upon removal from the water bath or plasma thawer, the surface temperature of the red cells is measured using an infrared scanner or a NIST certified thermometer and should be between 30 and 34 C.

### II. PROCEDURE

#### A. WATER BATH (BLUE-M, MODEL MW1140A)

1. Latex gloves must be worn throughout this procedure.
2. Turn on the power switch of the water bath located at the end of the water bath. Allow the water to warm to 42 C (approximately 1 hour). Switch on the circulating pump in the water bath used to thaw the frozen red cells. Allow the pump to run for 1-2 minutes, then check the water temperature to ensure that it has stabilized at 42 C.
3. Using freezer gloves, remove the box containing the red cells from the freezer. Record the time on the Deglycerolization Logsheet when the frozen red cells are placed in the water bath as the beginning of the deglycerolizing time period. Processing must be completed and the deglycerolized red cells must be placed in a 1-6 C refrigerator within 2 hours of removal from the -80 C freezer.
4. Open the freezing container and remove the unit of frozen red cells.
5. Thaw the unit still in its plastic overwrap by immersing it in the water bath. Place lead weights on top of the units so that the units remain submerged during the thawing procedure.

**NOTE:** The thawed red cells inside the plastic overwrap should remain in the water bath until they reach a temperature between 30 and 34 C. This will normally take approx. 45 minutes.

6. Remove the unit from the water bath and check the temperature of the unit using an infrared scanner. If the temperature of the unit is not between 30 and 34 C, replace the overwrapped unit back into the water bath. As described above, recheck the temperature every 5 minutes until the desired temperature is achieved.
7. Remove the unit from the water bath and dry off the overwrap. Tear open the overwrap and discard it. Wrap the thawed unit loosely in a disposable white towel. Check the bag for any breaks by gently compressing the unit in the towel, wiping the entire bag surface with the towel and then inspecting the towel for blood stains. The presence of blood stains on the towel is evidence of bag breakage, and the unit must be considered contaminated. Units suspected of being contaminated should be disposed of in compliance with local Standard Operating Procedures for the disposal of liquid-stored blood products.
8. The thawed glycerolized red cells are now ready for deglycerolization.

**B. PLASMA THAWER (THERMOGENESIS, MODEL MT204)**

1. Latex gloves must be worn throughout this procedure.
2. Turn on the power to the plasma thawer and allow the system to warm to 40 C ( $\pm$  1 C).
3. Using freezer gloves, remove the box containing the red cells from the freezer. Record the time on the Deglycerolization Logsheet when the frozen red cells are placed in the water bath as the beginning of the deglycerolizing time period. Processing must be completed and the deglycerolized red cells must be placed in a 1-6 C refrigerator within 2 hours of removal from the -80 C freezer.
4. Open the freezer container and remove the unit of frozen red cells.
5. Remove the plastic overwrap and place the unit into one of the pouches of the plasma thawer.



**NOTE:** The thawed red cells without the plastic overwrap should remain in the pouch of the plasma thawer until they reach a temperature between 30 and 34 C. This will normally take approx. 30-35 minutes.

6. Remove the unit from the plasma thawer. Wrap the thawed unit loosely in a disposable white towel. Check the bag for any breaks by gently compressing the unit in the towel, wiping the entire bag surface with the towel and then inspecting the towel for blood stains. The presence of blood stains on the towel is evidence of bag breakage, and the unit must be considered contaminated. Units suspected of being contaminated should be disposed of in compliance with local Standard Operating Procedures for the disposal of liquid-stored blood products.
7. The thawed glycerolized red cells are now ready for deglycerolization.

## DEGLYCEROLIZATION

### HAEMONETICS BLOOD PROCESSOR 115

#### I. INTRODUCTION

The Haemonetics Blood Processor 115 is a gravity flow, non-programmed, continuous-flow washing system which has a mixing platform of fixed oscillation rate and excursion distance integrally attached. The system is designed to wash 2 units of thawed red cells, intended for the same person, in a single bowl. After the red cells have been thawed, the plastic collection-freezing bag is secured to the platform on the Haemonetics 115 by means of adjustable magnetic mounting posts. The cells are first diluted once with 12% sodium chloride, and then twice with 0.9% sodium chloride-0.2% glucose solution, utilizing the shaking platform to ensure adequate mixing. After dilution of the red cells, the primary collection bag is removed from the platform and suspended in an inverted position on a support hook above the wash bowl and the red cells are permitted to flow into the spinning wash bowl until the first effluent is noted in the waste line. As soon as the waste appears, the flow of 0.9% sodium chloride-0.2% glucose solution is initiated. This solution flows simultaneously with the remaining red cells entering the bowl and then continues until a total volume of 1.5 liters has entered the bowl. At the completion of the wash cycle, the centrifuge is stopped and the deglycerolized red cells are syphoned from the wash bowl into a 600 ml pack of the quadruple red blood cell recovery bag system. The unit is labeled with the expiration date and time. Just before transfusion the washed red cells are concentrated by centrifugation, and the supernatant solution is removed to the integrally attached 600 ml transfer pack and discarded.

**NOTE:** At the present time, red cells not used immediately after washing may be stored at 4 C for up to 24 hours.

#### II. MATERIALS

##### CONSUMABLES

1. Double blood spike harness, washing bowl and waste bag shown in Figure 6 (Haemonetics 7497)
2. Dry quadruple RBC recovery bag system (Haemonetics 842)

3. 12% Sodium Chloride Solution (150 ml plastic bag) (Fenwal 4B7874); Each 100 ml contains: 12 g sodium chloride USP.

**NOTE: Only 50 ml of this solution are used for each unit of red cells.**

4. 0.9% Sodium Chloride-0.2% Glucose Solution (2-liter plastic bag) (Fenwal 4B7878). Each 100 ml contains: 200 mg dextrose (hydrous) USP, 900 mg sodium chloride USP.

**NOTE: Only 1.5 liters of this solution are used for each unit of red cells.**

### III. MACHINE SET-UP

Since the Haemonetics Blood Processor 115 operates on the principle of gravity flow, the heights at which the blood and solutions are hung will determine the flow rate. The four support hooks should be positioned as follows (Figure 7):

SOLUTION	HEIGHT	FLOW RATE
12% Sodium Chloride solution	16.0 inches* (middle solution hook)	Approx. 100 ml/min
0.9% Sodium Chloride 0.2% Glucose solution (dilution height)	31.5 inches* (uppermost solution hook)	Approx. 100 ml/min
0.9% Sodium Chloride- 0.2% Glucose solution (wash height)	9.5 inches* (lowermost solution hook)	Approx. 120 ml/min
Blood bag (Wash height)	3.5 inches* (blood bag hook)	Approx. 75 ml/min

\*Measured from the top of the mixer to the base of the hook (Figure 7).

Upon installation of the Haemonetics 115, the operator should check to see that the recommended heights actually yield the expected flow rates (see Quality Control Section). The solution hook should be adjusted so that the desired flow rate is achieved. Excessive flow rate can result in red cell spillage. Red cell spillage may occur when large units containing greater than the normal number of red blood cells are processed, and in such cases the wash solution should be lowered to reduce the flow rate until spillage ceases.

1. Gloves must be worn throughout this procedure. Remove the disposable wash set from its box and CLOSE ALL SIX SLIDE CLAMPS on the harness tubing (Figure 6). Check to see if all slide clamps were provided on the harness tubing and that all four bag spikes and the component bag receptor port are properly covered. Install bowl, harness, and waste bag on the machine according to the manufacturer's instructions (page 3-11 through 3-17). A copy of the manufacturer's instructions is included as part of this Standing Operating Procedure (see Appendix A). Also refer to the videotape procedure, produced by the Naval Blood Research Laboratory, for detailed instructions.
2. Remove the dry quadruple red blood cell recovery bag system from its box. CLOSE ALL FOUR SLIDE CLAMPS on the tubing, and aseptically insert the spike of the dry quadruple red blood cell recovery bag system (Haemonetics 842) into the component bag receptor port on the cell wash harness (Figure 6). Place the dry quadruple red blood cell recovery bag system on the hooks provided on the front of the cell wash stand.
3. Aseptically insert the spike on the blue color-coded harness line into the bag of 12% sodium chloride (Figure 6). Invert the bag and hang it on the middle solution support hook (Figure 7).
4. Aseptically insert the spike on the yellow color-coded harness line into the bag of 0.9% sodium chloride-0.2% glucose solution (Figure 6). Invert the bag and hang it on the uppermost solution support hook (Figure 7).

5. Insert the spike on the red color-coded harness line into the administration port of the bag of thawed red cells (Figure 6) and place the bag on the shaker.
6. Arrange the shaker magnets and the unit on the shaker platform so that the ports of the primary bag point toward the operator. The bag should be stretched flat so that the maximum surface area covers the shaker. This will insure proper mixing during the dilution steps. The blood bag label should face down so that the operator can observe mixing of the wash solution with the thawed red cells.

#### IV. DILUTION OF THE THAWED RED CELLS BEFORE WASHING

1. Using the factory suggested graduations as a guide, mark the bag of 12% sodium chloride solution at the level expected when 50 ml of the solution has been added to the unit (Figure 8).

**CAUTION:** Damage (gross hemolysis) to the red cells may occur if more than 50 ml of the 12% NaCl solution is added to the thawed unit.

2. **FIRST DILUTION:** Turn the shaker on, open the slide clamp on the tubing leaving the blood bag and open the slide clamp on the tubing leaving the 12% sodium chloride (Figure 7), and allow approximately 50 ml of this solution to enter the unit (approx. 30 seconds). Close both slide clamps and turn off the shaker. Allow the red cells to equilibrate with this solution for at least 2 minutes.

**NOTE:** Visually check the unit for signs of localized solution pooling as indicated by deep reddish-to-black-colored areas, caused by inadequate mixing. This can be remedied by repositioning the bag on the shaker platform before continuing the dilution process.

3. Using the factory suggested graduations as a guide, mark the 0.9% sodium chloride-0.2% glucose solution bag at the points where the solution level should be when one dilution of approximately 100 ml and a second dilution when an additional 150 ml have been added to the unit (Figure 8). Make a third mark on the bag (for the wash cycle) at a point 1250 ml below the 150 ml dilution mark (Figure 8).

**NOTE:** The total amount of this solution used is 1500 ml.

4. **SECOND DILUTION:** Turn the shaker on, unclamp the slide clamp on the tubing leaving the blood bag and the slide clamp on the tubing leaving the bag of 0.9% sodium chloride-0.2% glucose solution (Figure 6) and allow approximately 100 ml of this solution to enter the unit. Watch the unit for signs of localized solution pooling as in Step 2 above. Close both slide clamps. Turn the shaker off. Allow the red cells to equilibrate with this solution for at least 2 minutes.

**NOTE:** Flow rate should be no faster than 100 ml/minute. This can be estimated by timing the rate of solution level fall across the factory graduation marks. If the flow rate is too rapid, it can be reduced by lowering the height of the solution support hook.

5. **THIRD DILUTION:** Turn the shaker on, reopen the slide clamp on the tubing leaving the blood bag and the slide clamp on the tubing leaving the bag of 0.9% sodium chloride-0.2% glucose solution (Figure 6), and allow approximately 150 ml of this solution to enter the unit. Clamp the tubing leaving the 0.9% sodium chloride-0.2% glucose solution bag and close the clamp on the tubing leaving the blood bag (Figure 6). Turn the shaker off. Allow the red cells to equilibrate with this solution for at least 2 minutes.

**V. WASH CYCLE**

1. Remove the unit from the shaker platform. Insert the bottom grommet of the blood bag onto the blood bag support hook to permit the unit to hang in an inverted position (Figure 7).
2. Relocate the 0.9% sodium chloride-0.2% glucose solution bag from the uppermost to the lowermost solution support hook (Figure 7).
3. Check all tubing for occluding kinks and straighten as necessary. Check the tubing attached to the cell wash bowl; it must not touch the centrifuge.

**CAUTION:** Be sure that the waste tubing never becomes occluded during this procedure. Occlusion of the waste tubing may generate back pressure in the cell wash bowl which could cause the rotating seal to vent to atmosphere.

4. Check to make sure that the feed tube support arm properly engages the feed tube of the cell wash bowl and that the centrifuge cover is properly placed onto the cell washer. Turn centrifuge on.
5. Set timer for 5 minutes.

**NOTE:** If a power failure occurs when the centrifuge is on, **IMMEDIATELY** close all the slide clamps. This will prevent gross spillage of red blood cell into the waste bag. When power is re-established, wait for the centrifuge to spin for 1 to 2 minutes to insure resedimentation of the red cells in the bowl. Then, reopen the slide clamps to finish the procedure.

6. Open the slide clamp on the tubing leaving the blood bag and the slide clamp on the tubing entering the cell wash bowl (Figure 6) to permit the diluted red cells to enter the spinning bowl. Visually check the flow of red cells into the bowl. The flow rate into the bowl should be approximately 75 ml per minute. If the bowl fills

in less than 5 minutes, or if the first effluent appears in the waste tubing before the timer sounds, the flow rate is too fast and the red cells may spill into the waste bag during the wash cycle. If this occurs, lower the blood bag support hook. Normally, the bowl should fill in 7 to 10 minutes. If an extended fill time is observed, check the tubing for kinks or aggregate materials which may clog the tubing. See Quality Control Section for additional information.

**NOTE:** The flow of red cells from the blood bag can be checked by inverting the blood bag momentarily, allowing air from the blood bag to enter the tubing. If the air bubbles do not move through the tubing to the bowl, the flow has stopped. Check for an occlusion in the tubing. Straighten tubing kinks or squeeze the primary bag to dislodge microaggregate material.

7. As soon as the first effluent appears in the waste line, unclamp the tubing leaving the 0.9% sodium chloride-0.2% glucose solution bag to permit this solution to flow into the bowl along with the remaining diluted red cells. INSPECT THE INLET TUBING ATTACHED TO THE BOWL. THIS TUBING MUST ALWAYS CONTAIN RED CELLS AS LONG AS RED CELLS ARE DRAINING FROM THE BLOOD BAG. If the tubing appears void of red cells at this point, look for tubing kinks or aggregates and then reestablish the flow of red cells into the bowl.

**NOTE:** A pale pink tinting with free hemoglobin (hemolysis) in the effluent waste is normal at this point. Using the free hemoglobin reference scale as a guide, estimate the degree of hemolysis in the waste. The degree of hemolysis should be equal to or less than the number 6 at this point in the wash cycle. If color of the waste appears to be equal to or darker than the number 7, check to see if the proper volume of 12% NaCl solution was added to the unit, otherwise consult the Quality Control section.

8. When all the diluted red cells have been transferred from the bag to the bowl, clamp the tubing leaving the blood bag.



9. Check the flow rate of the 0.9% sodium chloride-0.2% glucose solution to be sure it does not exceed 120 ml/minute. The flow rate can be checked by timing the rate of fall of the solution across the factory graduation marks. One hundred mls will take 50 seconds to flow out of the bag at a rate of 120 ml per minute.

**NOTE:** An excessive flow rate will result in spillage of the red cells into the waste during the wash cycle. Spillage is detected by the examination of the waste which exits the cell wash bowl. If red cell spillage occurs, the red color in the waste line will appear cloudy red as opposed to transparent red. If spillage is observed, lower the height of the blood bag and the bag of 0.9% NaCl-0.2% Glucose.

10. Normally, the pale tinge of hemolysis in the effluent waste line should disappear after delivery of 1,000 to 1,200 ml of 0.9% sodium chloride-0.2% glucose solution in the wash cycle.

**NOTE:** If the red color of effluent has not disappeared when 1,000-1,200 ml of wash solution has been used, check the waste effluent for spillage of red cells and reduce the flow rate as necessary.

11. Clamp the tubing leaving the wash solution and the tubing leaving the bowl when the level of 0.9% sodium chloride-0.2% glucose solution reaches the 1,500 ml point marked on the solution bag in IV-Step 3 above. IT IS IMPORTANT THAT A TOTAL OF 1500 ML OF WASH SOLUTION IS USED TO INSURE THAT THE RESIDUAL GLYCEROL LEVEL IS BELOW 1%.

**NOTE:** The color of the waste should be equal to or less than the number 3 using the free hemoglobin reference scale at the completion of this procedure. Continued discharge of hemolysis after this point indicates that the unit of blood is washing abnormally and should be studied prior to transfusion (See Quality Control section). There is very little variation from unit to unit with this wash protocol.

12. Turn the centrifuge off.

13. Once the bowl has stopped rotating, open the two clamps on the tubing between the bowl and the dry quadruple RBC recovery bag system (Haemonetics 842) to allow the red cells to flow into one of the empty 600 ml transfer packs.
14. Squeeze air from the waste bag into the bowl to force the washed red cells out of the bowl and establish a syphon flow of red cells into the 600 ml pack of the dry quadruple RBC recovery bag system. When syphon flow begins, stop squeezing the waste bag. DO NOT SQUEEZE AIR OUT OF THE WASTE BAG AND INTO THE DRY QUADRUPLE RBC RECOVERY PACK. Air bubble gaps will appear in the tubing between the bowl and the 600 ml pack of the dry quadruple RBC recovery bag system, and the syphon flow will stop when the bowl has been emptied.

**NOTE:** As the bowl drains, sterile air trapped in the waste bag leaves the waste bag and enters the bowl. **CAUTION: WASTE SOLUTION SHOULD NOT RETURN TO THE BOWL.**

15. Remove the 600 ml transfer pack containing the red cells from the cell washer stand. Turn the transfer pack to the upright position and squeeze the trapped air from the transfer pack into the bowl. Continue squeezing to fill the integral tubing with red cells. Clamp the tubing between the bowl and the 600 ml pack of the dry quadruple RBC recovery bag system.
16. Affix unit number, ABO/Rh and deglycerolization facility ID labels to the label on the 600 ml pack of the dry quadruple RBC recovery bag system, and note the date washed and expiration date and time on the label (24 hours from the time the frozen red cells were placed in the water bath for thawing) (Figure 9).
17. Using the Sebra integral tubing sealer, seal the tubing three times between the bowl and the deglycerolized unit leaving as much integral tubing attached to the 600 ml transfer pack as necessary.

**NOTE:** To use the Sebra heat sealer, place the tubing into the sealing head. Squeeze the sealing head handle completely. A pink light will illuminate. Release the handle when the light goes off. **DO NOT RELEASE THE HANDLE WHILE THE LIGHT IS ON.** Consult the users manual for additional instructions.

18. Detach the unit of deglycerolized red cells with the integrally attached transfer pack from the harness, by cutting the middle one of the three heat seals. Make sealed crossmatch segments with the Sebra sealer as required. One empty transfer pack with an integrally attached transfer pack will remain attached to the cell wash harness for processing of the second unit of blood.
19. Record the time that the deglycerolized red cells are placed into a 1-6 C refrigerator as the end of the deglycerolizing time period. Compare this time to the beginning time recorded in Step 3 of the Thawing Procedure to verify that deglycerolization was accomplished within the required 2-hour time period.

**CAUTION:** If at any time during this procedure the cell wash disposable system becomes vented to room air, the unit and disposable set must be discarded.

**PROCEDURE CONTINUATION FOR SECOND UNIT**

1. Remove the overwrap from the second bag of 0.9%-NaCl 0.2% Glucose solution. Aseptically, withdraw the yellow color-coded spike from the used 2-liter bag of 0.9% sodium chloride-0.2% glucose solution and discard.
2. Aseptically insert the yellow color-coded spike into the full 2-liter bag of 0.9% sodium chloride-0.2% glucose solution and reposition the bag on the uppermost solution hook.
3. Remove the empty primary bag (first unit processed) from the blood bag support hook. Insert the remaining red color-coded harness spike into the administration port of the second unit of thawed glycerolized red cells to be deglycerolized, and place the second unit on the shaker. Place the empty primary bag (first unit processed) on the shaker, underneath the second unit to be deglycerolized. Arrange the shaker magnets and the unit on the shaker platform so that the ports of the primary bag point toward the operator. The bag should be stretched flat so that the maximum surface area covers the shaker. This will insure proper mixing during dilution steps. The blood bag label should face down so that the operator can observe mixing of the wash solution with the thawed red cells.

**NOTE:** The used blood bag spike must remain attached to the empty primary bag (the first unit washed) throughout the processing of the second unit.

4. Continue processing the second unit by repeating IV Steps 1-5 and V Steps 1-19.

**NOTE:** During the washing of the second unit, during the pre-dilution phase, be sure to unclamp the correct slide clamp which enters the blood bag of the second unit.

## VI. STORAGE AND ISSUE

Since units washed in the same disposable bowl must be transfused to the same recipient, secure the two units of deglycerolized red cells with an elastic band or tie tag during storage at 4 C.

**NOTE:** Sister unit numbers should be entered into the Defense Blood Standardization System (military use only)

Place the deglycerolized red cells (hematocrit approximately 40%) into a refrigerator maintained at 1-6 C for up to 24 hours (24 hours from the time the frozen red blood cells were placed in the water bath for thawing).

At the time of transfusion, the red cells are concentrated by centrifugation at  $22 \pm 2$  C at 2982 X g for 4 minutes (Table 1), and the supernatant 0.9% sodium chloride-0.2% glucose solution from the unit is expressed completely into the integrally attached 600 ml transfer pack of the dry quadruple RBC recovery bag system. Each unit has a hematocrit value of about 85 V%. Heat seal the tubing and detach the 600 ml transfer pack containing the supernatant.

### HAEMONETICS CELL WASH HARNESS

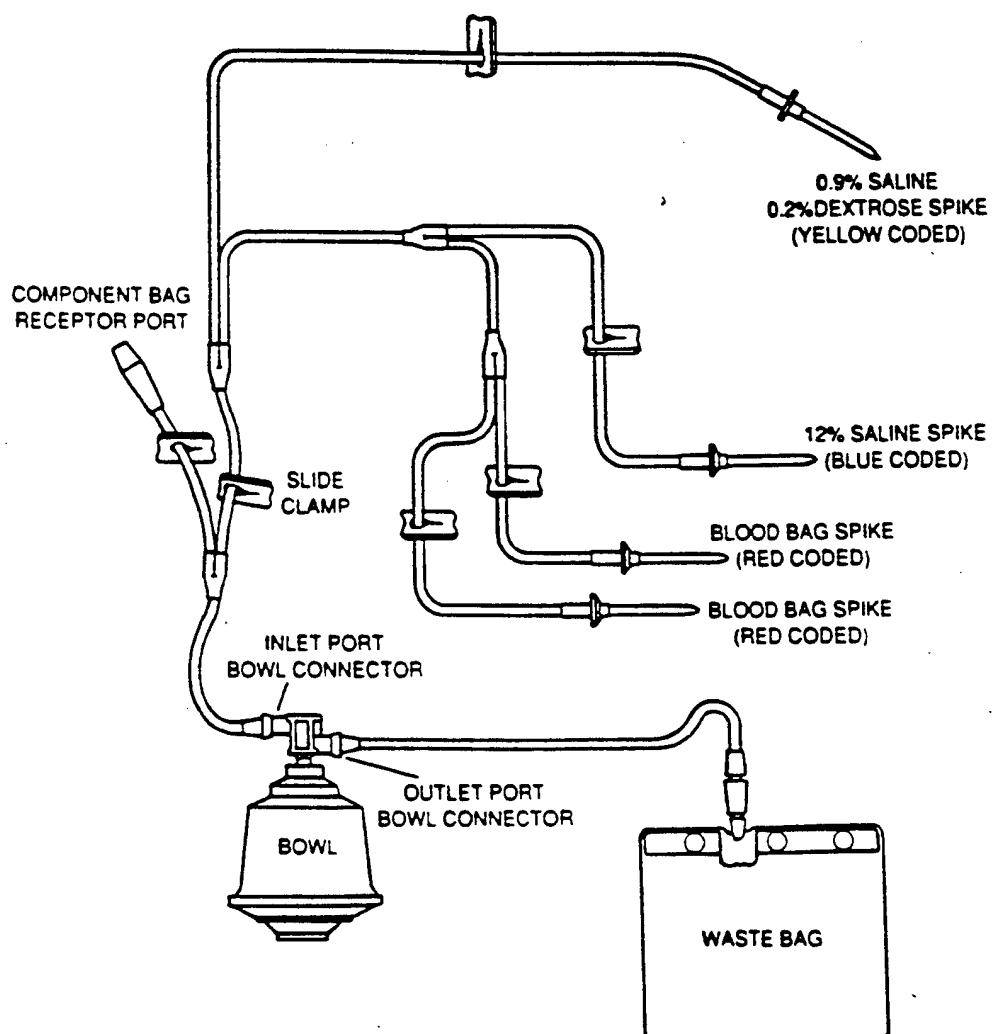


FIGURE 6

# Post-Dilution Heights

# Pre-Dilution Heights

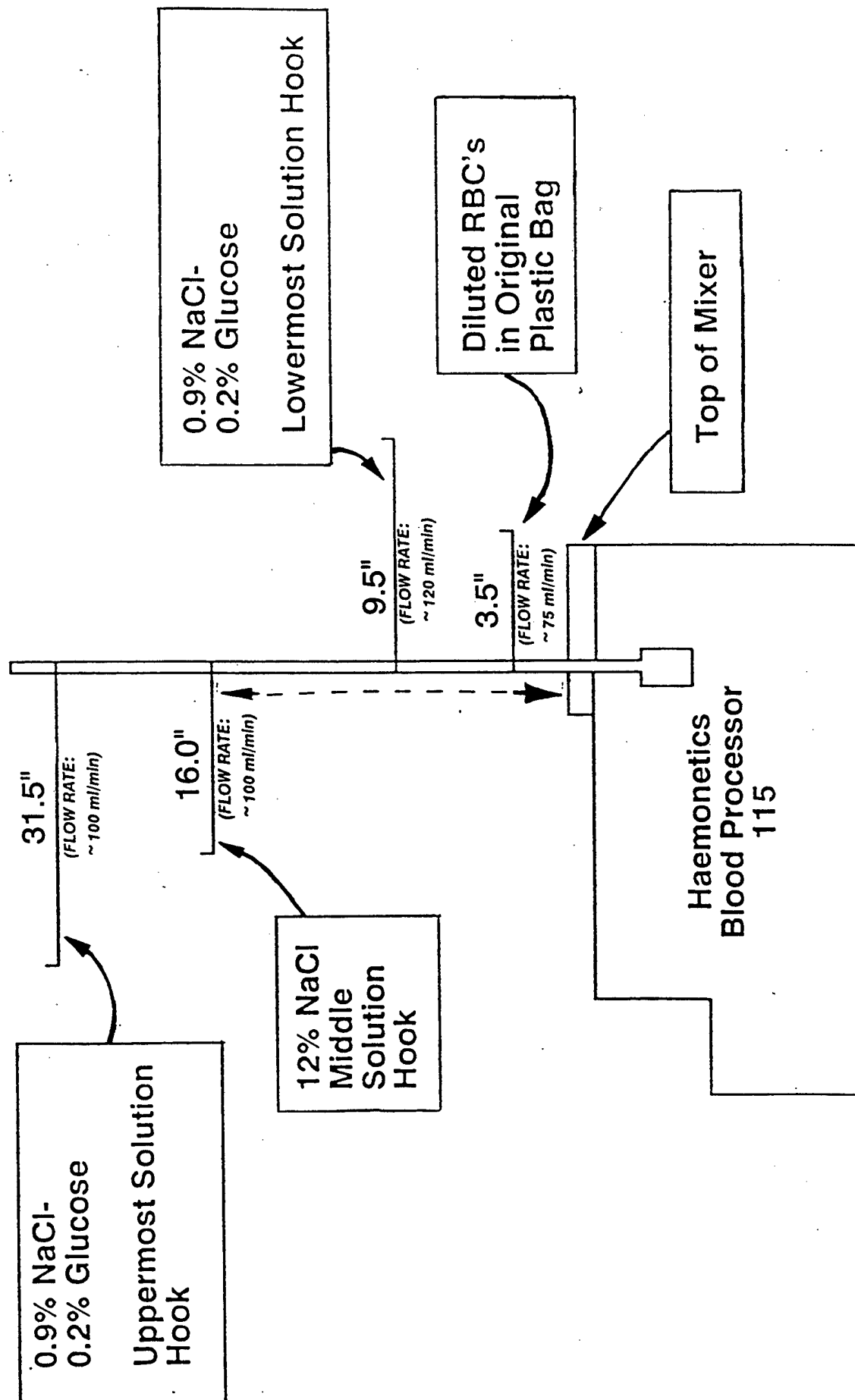


FIGURE 7

# VOLUMES OF SOLUTIONS USED IN DILUTION AND WASHING OF GLYCEROLIZED RED BLOOD CELLS

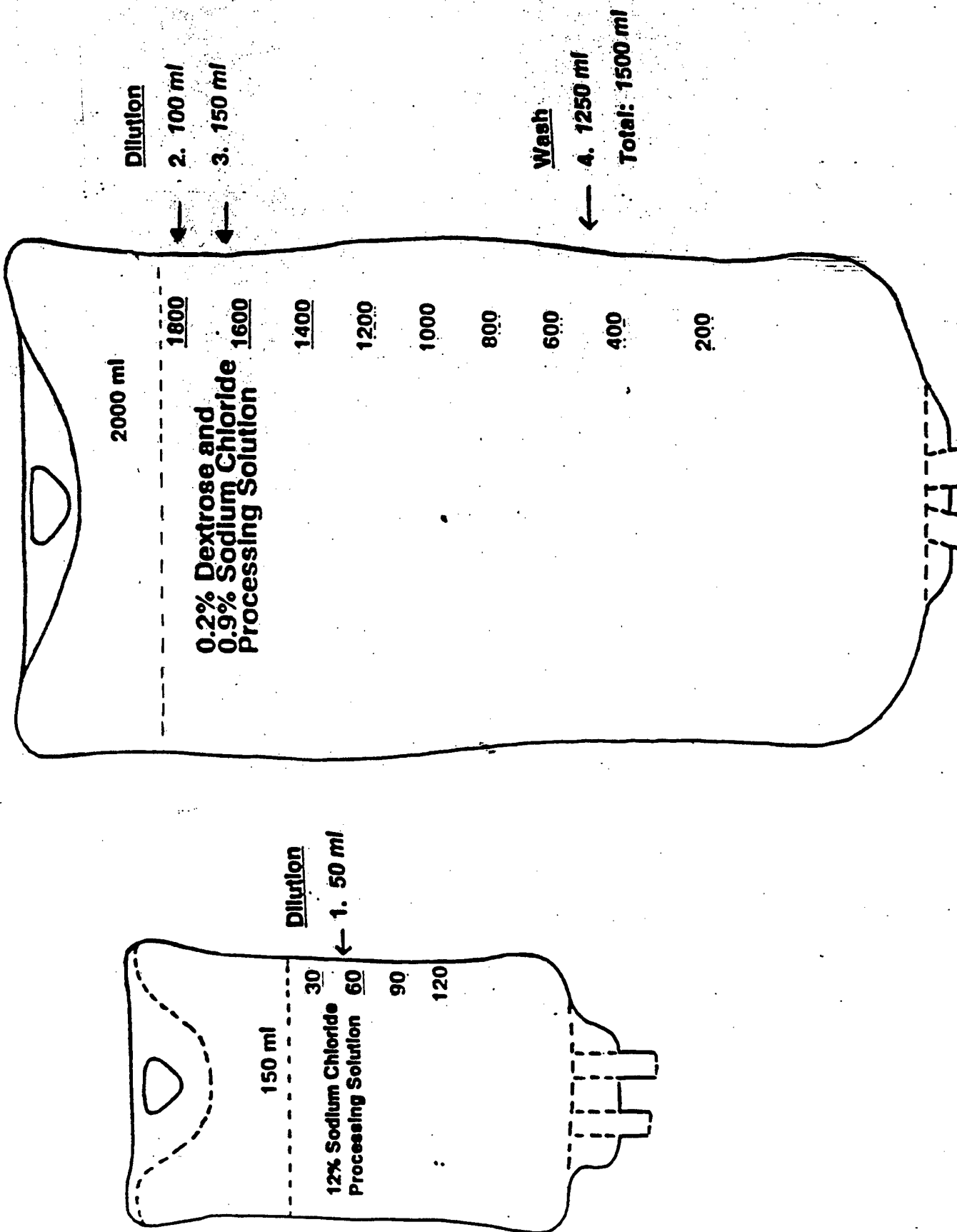


FIGURE 8



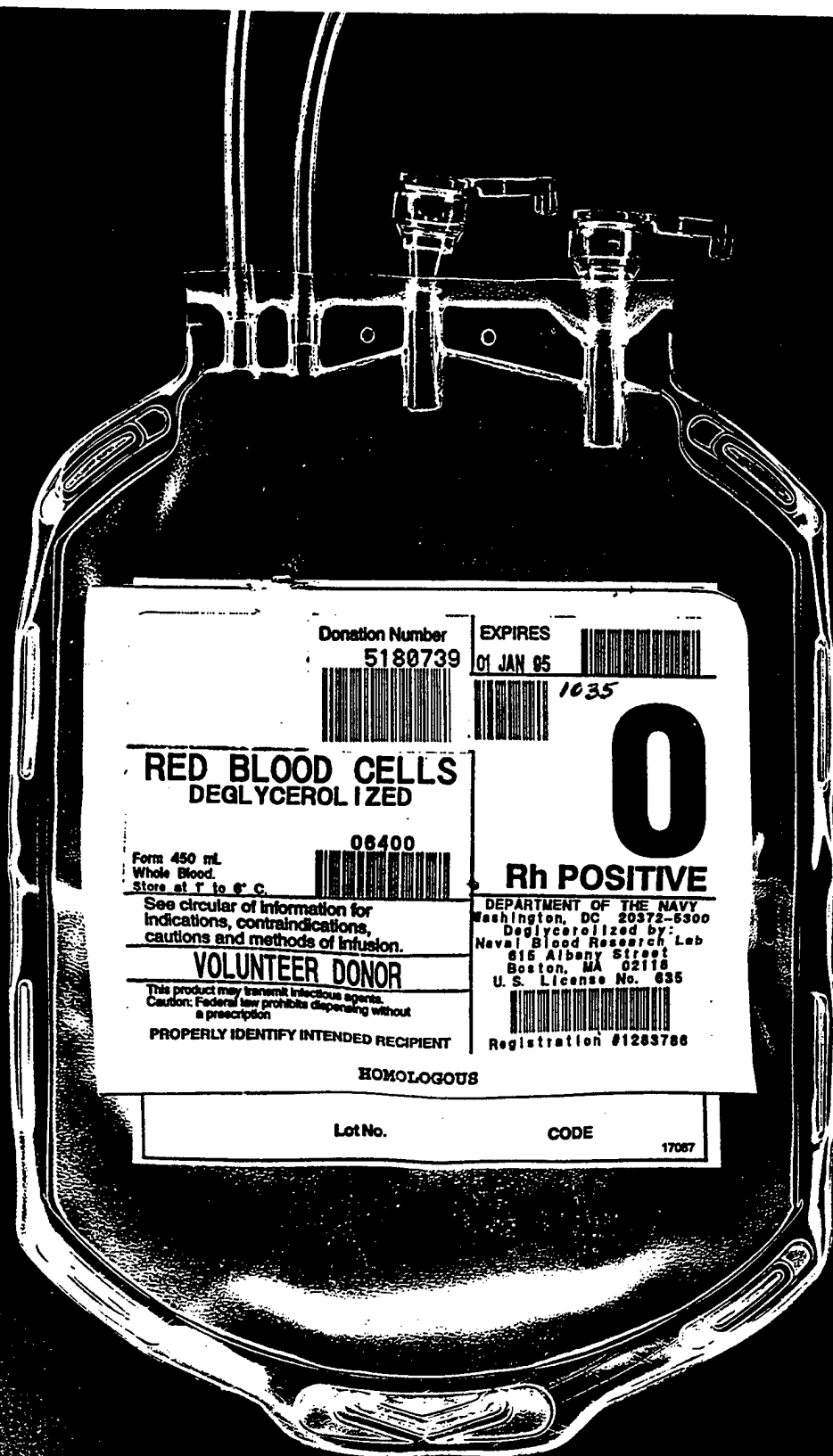


FIGURE 9.

Revised 4/97

**Red Blood Cell Deglycerolization Worksheet**  
**Naval Blood Research Laboratory**  
**Boston University School of Medicine**  
**615 Albany St., Boston, MA 02118**

UNIT # \_\_\_\_\_ DATE \_\_\_\_\_

SISTER UNIT # \_\_\_\_\_

WASHED FOR \_\_\_\_\_

AUTOLOGOUS  
TRANSFUSION YES \_\_\_\_\_ NO \_\_\_\_\_

STUDY \_\_\_\_\_

AGE PRE FREEZE \_\_\_\_\_

REJUVENATION YES \_\_\_\_\_ NO \_\_\_\_\_

**THAW METHOD:**

42 C WATER BATH \_\_\_\_\_ MIN. 40 C THERMOGENESIS \_\_\_\_\_ MIN.  
 MEASURED TEMP. \_\_\_\_\_ C (INFRARED SCANNER OR NBS THERMOMETER)

WASH TIME: IN WATER BATH/THERMOGENESIS \_\_\_\_\_  
 WASHED UNIT IN 4 C REFRIG. \_\_\_\_\_  
 TOTAL TIME \_\_\_\_\_  
 WITHIN 2 HOURS? YES \_\_\_\_\_ NO \_\_\_\_\_

WASH INSTRUMENT: HAEMONETICS 115 \_\_\_\_\_ IBM \_\_\_\_\_ SERIAL# \_\_\_\_\_  
 BOWL SEAL: INT \_\_\_\_\_ EXT \_\_\_\_\_

	MFG	LOT	EXP. DATE
WASH HARNESS	_____	_____	_____
12% NACL	_____	_____	_____
0.9% NACL/0.2% GLUCOSE	_____	_____	_____
DRY QUAD PACK	_____	_____	_____

**Q.C.**

GROSS BLOOD AND BAG WEIGHT  
 WITHIN 30 g OF WT ON FRZ SHEET \_\_\_\_\_ ACCEPT. \_\_\_\_\_ NOT ACCEPT. \_\_\_\_\_  
 (RANGE: 320-610 g)

SPILLAGE YES \_\_\_\_\_ NO \_\_\_\_\_ ACCEPT. \_\_\_\_\_ NOT ACCEPT. \_\_\_\_\_

HEMOLYSIS YES \_\_\_\_\_ NO \_\_\_\_\_ ACCEPT. \_\_\_\_\_ NOT ACCEPT. \_\_\_\_\_

BREAKAGE YES \_\_\_\_\_ NO \_\_\_\_\_ WHERE \_\_\_\_\_

BACTERIAL CULTURE: AEROBIC \_\_\_\_\_ AEROBIC \_\_\_\_\_ NG=NO GROWTH  
 DATE CULTURE READ: \_\_\_\_\_

DEGLYCEROLIZED BY: \_\_\_\_\_

RESULTS ACCEPTED BY: \_\_\_\_\_ DATE: \_\_\_\_\_

## QUALITY CONTROL

### I. INTRODUCTION

All units are to be inspected for breakage and for wet ports, and the effluent (waste solution) observed throughout the wash cycle. We recommend that 1-2 units of red cells be used for quality control testing each month. Units used for quality control testing should not be used in vivo, even when results are satisfactory. When personnel are being trained to freeze, thaw and wash red cells, the units should be quality-controlled. Recommended quality control procedures are outlined below.

### II. MATERIALS

#### CONSUMABLES:

1. Sampling site coupler (Fenwal 4C2405)
2. Alcohol swab, 70% (B-D 6894) (3);
3. Blood agar plates (2)
4. BBL Septi-Check system (Fisher RD43231)
5. Syringe, 30 ml (B-D 5662) with 16 gauge needle (B-D 5198)
6. Syringe, 20 ml (B-D 5661) with 16 g needle (B-D 5198)
7. Plastic test tube (Falcon 2059)
8. Plastic test tubes (Falcon 2063) (2)
9. Universal fit pipet tip (Costar 4865 (yellow))
10. Universal fit pipet tip (Costar 4867 (blue))
11. Methanol (Fisher A-452)
12. IL 943 flame photometer standard, calibrator, cesium diluent, and sample cups
13. Cyanmethemoglobin reagent (Boehringer-Mannheim 116A0000) and cyanmethemoglobin standard (Fisher 2370-19)
14. Osmometer standard (290 mOsm/kg)
15. 4 X 4 gauze or Kimwipes.

16. Chemistrip 4 The OB (urine) (Boehringer-Manheim 417144)
17. Transfer pipet for ABBE refractometer
18. Glass culture tube, 13X100 mm (Kimax 45048)

### III. VISUAL OBSERVATION AND INSPECTION (ALL UNITS).

- A. BREAKAGE. Discard any unit that shows evidence of breaks or unintended openings at any point during processing.
  1. Check each thawed unit for container breaks by gently compressing the unit against a white disposable towel, wiping the entire unit surface after compression, and examining the towel for blood stains.
  2. Visually inspect the wash harness, wash chamber, waste bag and interconnecting tubing for evidence of breaks or leaks before, during, and after the deglycerolization process.
- B. WET PORTS. Before opening the port seal to connect the thawed unit to wash harness, visually inspect the port areas for any evidence of residual water droplets. Carefully and thoroughly wipe these areas dry with a clean cloth as necessary.
- C. OBSERVATION OF EFFLUENT. Check the appearance of the waste solution through the wash cycle for signs of excessive hemolysis or of red cell spillage.
  1. Hemolysis. At the beginning of the wash cycle the supernatant manifests a pale pink tinge which fades until it disappears after about 1200 ml of wash solution is used. If signs of excessive hemolysis persist, the unit must be studied to determine whether the unit is safe for transfusion (see below). Hemolysis results from a freeze-thaw lesion or from mishandling during red cell washing, and the following should be checked:
    - a. Check freezer temperature charts during the storage period.

- b. Check to see if the units that exhibit hemolysis were frozen at the same time or by the same person. Isolate any suspect units and evaluate and discard as necessary.
- c. Confirm technician understanding of pre-glycerolization handling, the three glycerolization steps and of the need for proper manual mixing of the glycerol with the red cells during the final addition, i.e., use of the table provided to determine the volume of glycerol to red cell weight.
- d. Confirm technician understanding of temperature requirements of the glycerol and red cells.
- e. Check accuracy of balance used for glycerolization.
- f. Check the gross weight and hematocrit of the thawed unit using the microhematocrit method. Units which exhibit gross weights greater than 500 grams and hematocrit values of less than 45 V% may wash poorly. Check the centrifugation procedure used to concentrate the glycerolized red cells to ensure that the unit is centrifuged at 1248 X g for 10 minutes. The brake on the centrifuge must be set at zero during centrifugation of the glycerolized red blood cells. All the visible supernatant solution is removed to achieve a hematocrit value of  $60 \pm 5$  V%.
- g. Confirm technician understanding of the predilution requirements prior to deglycerolization.
- h. In units with poor freeze-thaw and freeze-thaw-wash recovery values, studies should be done to determine whether or not the poor in vitro results were due to the quality of the red blood cells that were frozen. Sickle trait red blood cells (SA), hereditary spherocytosis (HS), paroxysmal nocturnal hemoglobinuria (PNH) red blood cells, and red blood cells with glucose-6-phosphate dehydrogenase deficiency do

not tolerate the freeze-thaw and freeze-thaw-wash recovery procedures. Red blood cells with poor freeze-thaw and freeze-thaw-wash recovery values with no apparent reason should be tested for these red blood cell abnormalities.

2. Spillage. When washing is performed using continuous-flow centrifugation, intact red cells can be observed in the effluent waste line. Spillage of intact red cells looks similar to hemolysis except that when intact red cells are present, the effluent appears cloudy red whereas when hemolysis is present, the effluent is transparent with a pink tinge. To detect whether hemolysis or loss of intact red cells is present, the effluent must be inspected against a white background. Spillage of red cells into the waste not only represents a loss of red cells from the unit but may also mask the presence of supernatant hemoglobin in the waste. The principal cause of spillage is the presence of too many red cells in the unit at the time of glycerolization. The weight of the red cells must be controlled during the collection of the blood to ensure that no more than 450 ml of blood is collected. Red cell spillage can also occur if the proper wash solutions are not used and if the spindle speed of the Haemonetics 115 is too slow. The following action is recommended if red cell spillage occurs:
  - a. Isolated unit spillage. Gradually lower the brackets supporting the sodium chloride-glucose solution and red cells until spillage ceases; these units are acceptable for transfusion as long as they meet all other criteria. Units in which spillage persists should be studied further to determine whether they are suitable for transfusion.
  - b. Recurrent and uncontrollable spillage.
    - (1) Check scale used to weigh units prior to glycerolization;
    - (2) Confirm technician understanding of glycerolization process;

- (3) Check spindle speed of 5800 rpm of the cell washing bowl using a hand-held tachometer;
- (4) Check labels and composition of wash solution.

**V. STERILITY (TRAINING UNITS AND MONTHLY QUALITY CONTROL UNITS)**

After the unit has been deglycerolized, a sample of the red cells is obtained by inserting a sampling site coupler (Fenwal 4C2405) into one of the entry ports of the 600 ml dry quad pack containing the red cells. Aseptically remove a 20.5 ml sample with a 30 ml syringe and 16 gauge needle for testing as follows:

1. Place a drop of red cells on each of two plates in the 4 quadrants of a blood agar plate (aerobic) and tilt the plate to allow each drop to streak each quadrant (0.5 ml sample is required).
2. The remainder of the sample will be put into the BBL Septi-Check system (Fisher #RD43231). This system consists of 2 vials, one containing 70 ml of thioglycollate broth (aerobic) and the other containing 70 ml of tryptic soy broth (anaerobic). Aseptically place 10 ml of blood into each of the vials according to the instructions provided with the culture system.
3. Incubate the blood agar plates at 37 C for 3 days and the broth tubes at 37 C for 7 days; examine for growth.

**V. DETERMINATION OF RESIDUAL GLYCEROL (OSMOLALITY), SUPERNATANT HEMOGLOBIN, AND EXTRACELLULAR POTASSIUM LEVELS (TRAINING UNITS AND MONTHLY QUALITY CONTROL UNITS).**

**SAMPLE REQUIREMENTS:** Aseptically insert an 16-gauge needle of a 20 ml syringe through the sampling site coupler (previously used for obtaining a sample for sterility testing) and withdraw a 15 ml sample of the deglycerolized red cells from the 600 ml transfer pack. Remove the 16-gauge needle from the syringe and discard. (Follow local guidelines for needle removal and disposal procedures. Transfer a 12 ml sample of deglycerolized red cells into a plastic test tube (Falcon 2059). Transfer the remaining 3 ml sample into a plastic test tube (Falcon 2063).

**NOTE:** Do not transfer the blood from the syringe through the needle into the test tube. This may cause hemolysis.

Centrifuge the sample at 2200 X g for 10 minutes in a 22 C refrigerated centrifuge. Transfer the supernatant into another plastic test tube (Falcon 2063) using a transfer pipet. The supernatant is used to determine residual glycerol (osmolality or refractive index/refraction), supernatant hemoglobin, and extracellular potassium levels.

## **RESIDUAL GLYCEROL**

### **Osmolality**

1. Calibrate the osmometer (Fiske Model 2400) using the manufacturer's procedure manual.
2. Using a Gilson adjustable volume pipettor and pipet tip, transfer 20 microliters of supernatant solution into an osmometer cuvette and determine the osmolality of the sample. Osmolality should not exceed 400 mOsm/kg H<sub>2</sub>O to insure a residual glycerol level of less than 1 g% (Figure 10).

### **Refractive Index/Refraction**

#### **ABBE Refractometer:**

1. Turn on the ABBE refractometer (American Optics Corp. Model 10480) and the constant temperature water bath (Haake Model A80) and allow to equilibrate to 20 C.
2. Calibrate the equipment with a known liquid material (e.g., absolute methanol).
3. Using a disposable transfer pipet, transfer 2 drops of supernatant solution into the refractive prism surface.
4. Close the refractive prism and determine the refractive index of the sample according to the manufacturer's specifications. The refractive index should not exceed 1.3355 to insure a residual glycerol level of less than 1 g% (Figure 11).



**Hand Held Refractometer:**

1. For field use, a Cambridge Instruments hand-held refractometer (TS meter, Model 10400A) may be used to estimate the residual level of glycerol. The refractometer contains a liquid prism which is self-temperature correcting. The meter has three scales; urine specific gravity, serum or plasma protein, and refraction. The refraction scale should be used; refraction is a mathematically derived value from the refractive index.
2. Using a disposable transfer pipet, transfer a sample of supernatant solution into the measuring prism, as described in the manufacturer's instruction manual.
3. Hold the instrument up to a light source (e.g., fluorescent light, window). Focus the eyepiece and determine the refraction value of the sample according to the manufacturer's instructions. The refraction value should be less than 30 to insure that the glycerol level is less than 1 g% (Figure 12).

**EXTRACELLULAR (SUPERNATANT) POTASSIUM**

1. Calibrate the IL 943 flame photometer using the flame standard 140 mEq/L Na<sup>+</sup>/5 mEq/L K<sup>+</sup>, according to the manufacturer's instructions.
2. Using a Gilson adjustable volume pipettor, add 300 microliters of supernatant into the sample cup of the flame photometer and measure the extracellular potassium level. The extracellular potassium level should not exceed 1.5 mEq/L on the day of washing.

**SUPERNATANT HEMOGLOBIN****SPECTROPHOTOMETRIC METHOD**

1. Set the Spectronic spectrophotometer at a wavelength of 540 nm.
2. Prepare a standard curve using a total hemoglobin standard kit (Sigma 525-A) according to the manufacturer's instructions. Included in the kit are: Drabkin's reagent, 30% BRIJ-35 solution, and a lyophilized hemoglobin standard (18 g%).

- A. Reconstitute the Drabkin's reagent (one vial) with 1000 ml of distilled water. Add 0.5 ml of the 30% BRIJ-35 solution. The Drabkin's solution may be stored at room temperature (18-26 C) in an amber bottle for up to 6 months.
- B. Reconstitute the lyophilized hemoglobin standard with 50 ml of Drabkin's solution to prepare an 18 g% solution.
- C. Pipet the following solutions to prepare the standard curve:

TUBE#	HEMOGLOBIN SOLUTION (ml)	DRABKIN'S SOLUTION (ml)	HEMOGLOBIN CONCENTRATION (g%)
1	0.0	6.0	0.0
2	2.0	4.0	6.0
3	4.0	2.0	12.0
4	6.0	0.0	18.0

NOTE: These diluted standards are stable for as long as 6 months when stored tightly capped, in the dark at 4 C.

- D. Place tube 1 into the spectrophotometer and zero the absorbance value. Read and record the absorbance values for tubes 2 through 4.
- E. Plot a calibration curve (absorbance values vs hemoglobin concentration). The curve should be linear and pass through the origin.
- F. Using the standard curve, calculate the extinction coefficient, K, as follows:  
  

$$\text{Hemoglobin Concentration (g\%)} = (K) (\text{Absorbance})$$
- G. Calculate the average K for using the three hemoglobin standard solutions (6.0, 12.0, 18.0 g%).
- H. Total hemoglobin measurements are performed using 0.02 ml of whole blood diluted with 5.98 ml of Drabkin's reagent (1:251 dilution).

- I. Measure the supernatant hemoglobin concentration using a 0.3 ml sample in 4.7 ml of Drabkin's reagent. The overall increase observed is 18-fold for the supernatant hemoglobin samples. The following formula is used to construct the supernatant hemoglobin standard curve:

$$\begin{array}{lcl} \text{Supernatant} & & \\ \text{Hemoglobin} & = & \frac{(K)(\text{Absorbance})(1000 \text{ mg/gram})}{18} \\ \text{Concentration} & & \end{array}$$

3. Using a Gilson adjustable pipettor, pipet a 0.3 ml sample of supernatant and dilute the sample with 4.7 ml of Drabkin's reagent into a 13X100 mm Kimax glass culture tube. Mix and equilibrate for at least 2 minutes for the reaction to occur. Measure the absorbance value for the sample using the Drabkin's reagent solution as the blank.
4. Refer to the supernatant hemoglobin standard curve and determine the hemoglobin concentration of the sample (mg%). The supernatant hemoglobin concentration of the day of washing should be less than 200 mg%.

**CHEMISTRIp METHOD** (Boehringer Mannheim Corp. Chemistrip 4 The OB urine test strip, Cat. No. 417144)

NOTE: The Chemistrip Method is recommended for all units of red cells washed. However, if a spectrophotometer is available, the Chemistrip Method should not be used in lieu of the spectrophotometric method for measurement of supernatant hemoglobin for quality control testing each month.

1. Prepare the supernatant sample (see Page 46).
2. Briefly (no longer than 1 second) dip the test strip into the supernatant sample. Ensure that the chemically impregnated patches on the test strip are totally immersed in the sample.
3. Draw the edge of the strip along the rim of the test tube to remove excess sample.
4. Turn the test strip on its side and tap once on a piece of absorbent paper to remove any remaining sample and to prevent the possible mixing of chemicals.

5. Wait 60 seconds, then visually compare the protein color patches on the test strip to the color scale printed on the vial label. The protein visual color scale bears five color patches, ranging from light yellow to dark green:

The first patch, designated as negative, indicates a supernatant hemoglobin value of approximately 16 mg%, measured using the spectrophotometric method.

The second patch, designated as trace, indicates a supernatant hemoglobin value of approximately 45 mg%, measured using the spectrophotometric method.

The third patch, designated as +30, indicates a supernatant hemoglobin value of approximately 96 mg%, measured using the spectrophotometric method.

The fourth patch, designated as ++100, indicates a supernatant hemoglobin value of approximately 221 mg%, measured using the spectrophotometric method.

The fifth patch, designated as +++500 mg/dl, indicates a supernatant hemoglobin value of approximately 428 mg%, measured using the spectrophotometric method.

6. Note the test result on the recovery sheet. No further calculations are necessary.

#### VI. ESTIMATION OF FREEZE-THAW-WASH RECOVERY (%) (TRAINING UNITS AND MONTHLY QUALITY CONTROL UNITS).

Total volume of waste solution. Measure the total volume of waste solution using a graduated cylinder. Usually, the total volume of waste solution is 1,500 ml/unit.

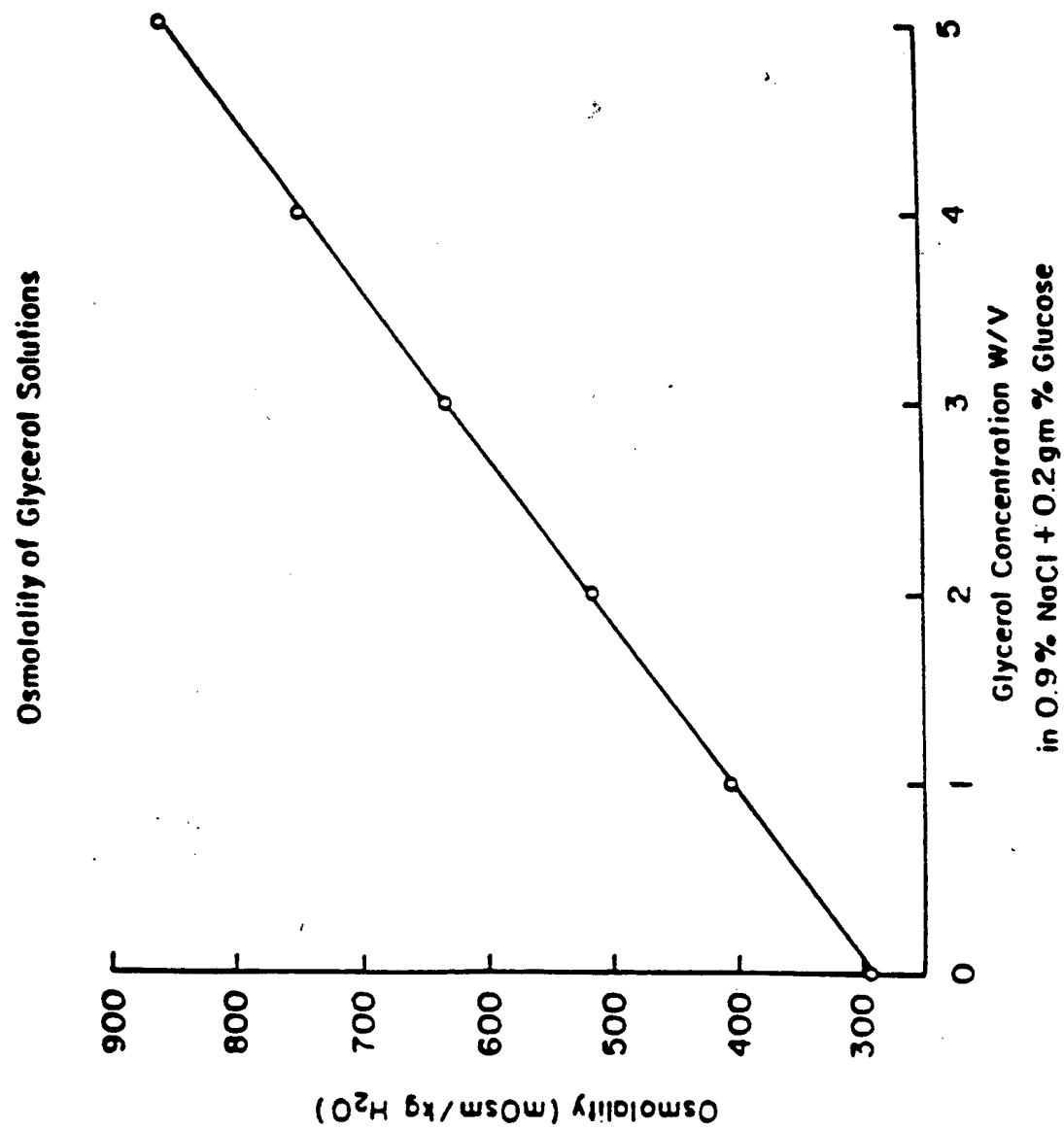
Hemoglobin concentration. Obtain a 5 ml sample from the waste bag and, using the same method described for the measurement of supernatant hemoglobin (mg%), determine the hemoglobin concentration. The hemoglobin concentration in the waste (mg%) multiplied by the total volume of waste (ml), divided by 100,000, will equal the total grams of hemoglobin lost. Since one unit of red cells contains 60-65 g of hemoglobin, the loss of 9 g of hemoglobin or less in the waste will ensure a total hemoglobin recovery of 85% or better.

**VII. VERIFICATION OF FLOW RATE OF 12% NaCl AND 0.9% NaCl-0.2% GLUCOSE WASH SOLUTIONS AND DILUTED RED CELLS (EVERY 6 MONTHS)**

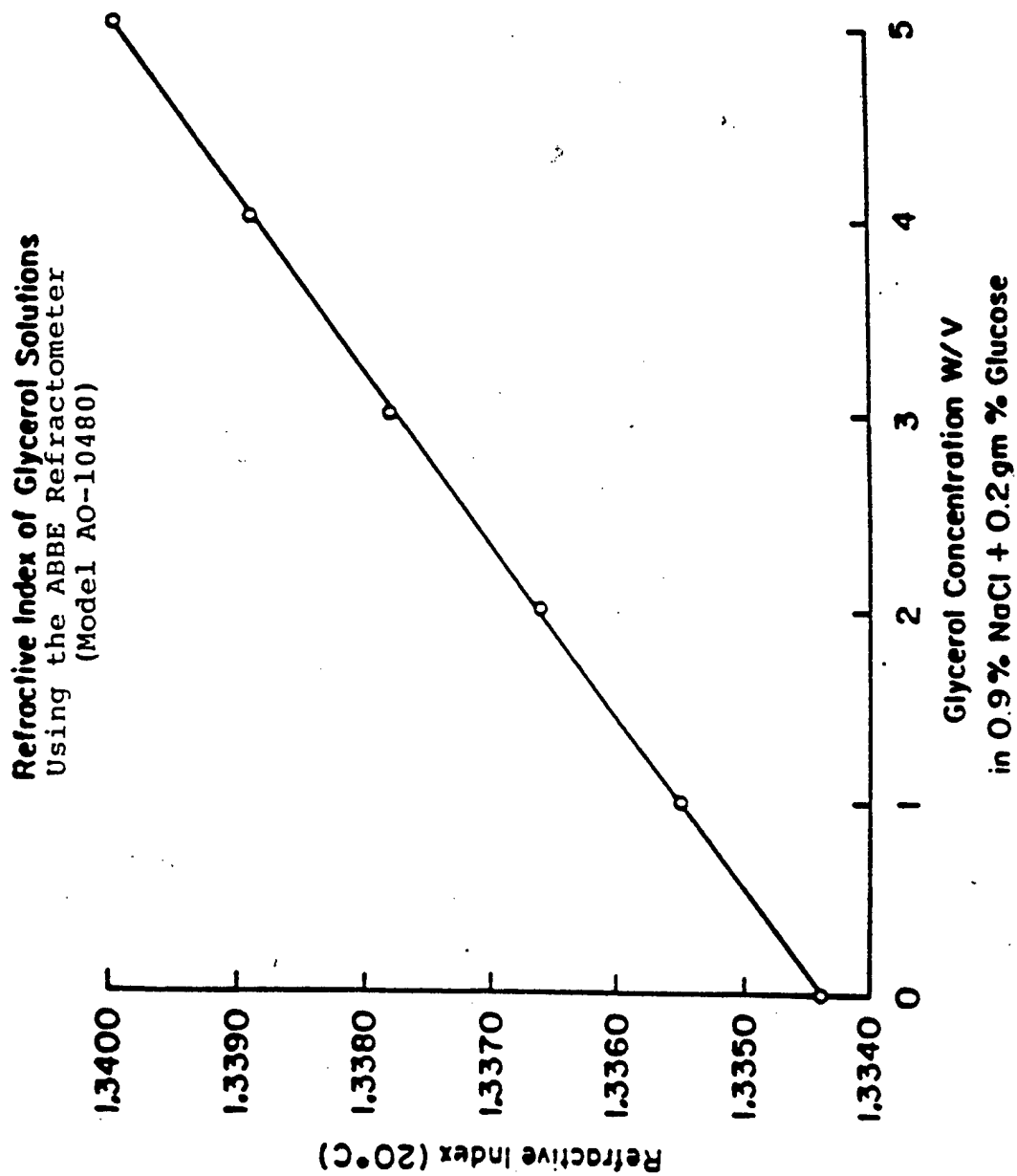
The flow rates of the 12% NaCl and 0.9% NaCl-0.2% glucose wash solutions and of the diluted red blood cells should be checked every 6 months. The recommended flow rates are as follows:

12% NaCl	100 ml/minute
0.9% NaCl-0.2% glucose (pre-dilution)	100 ml/minute
0.9% NaCl-0.2% glucose (post-dilution)	120 ml/minute
Diluted RBC	75 ml/minute

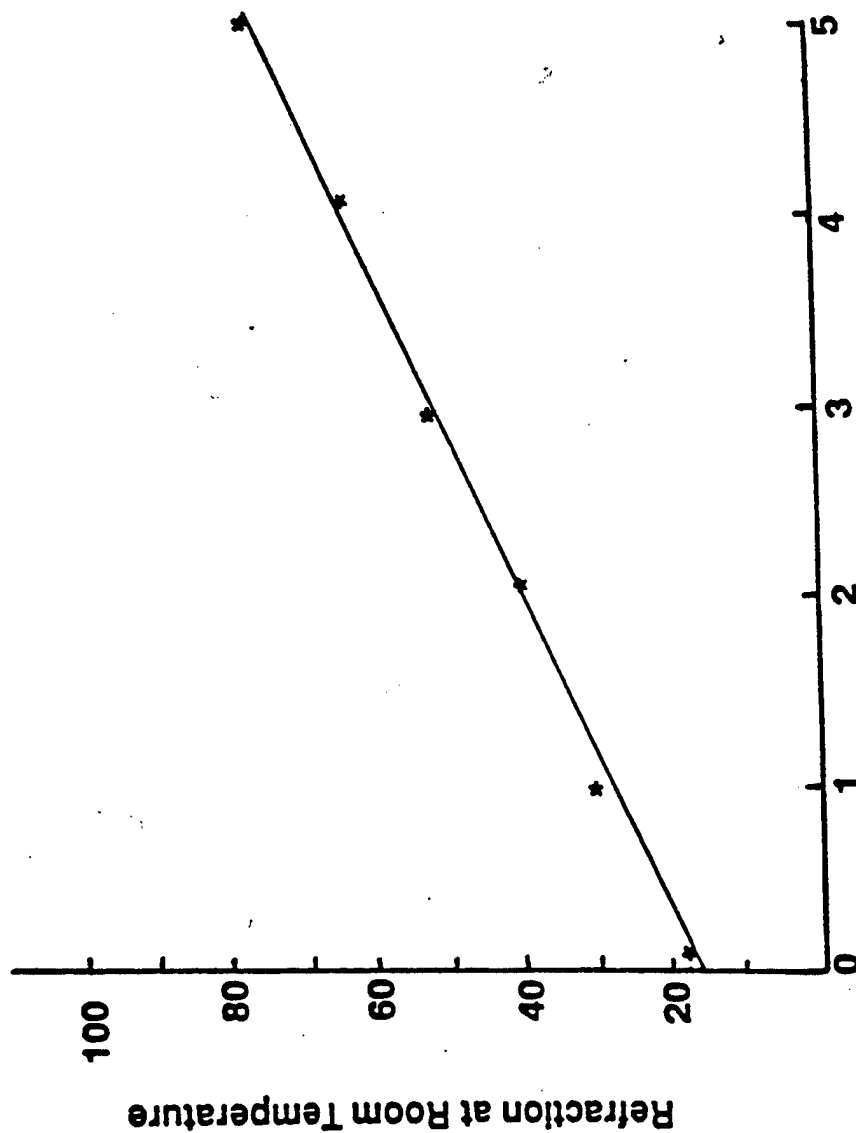
Hang the wash solutions and a bag of saline (in lieu of diluted red cells) on the appropriate hooks. Using a stopwatch, time the delivery of the solutions into an empty plastic bag. Adjust the hooks to achieve the proper flow rates as outlined above, if necessary. Make the appropriate corrections in your Standard Operating Procedure Manual.



**FIGURE 10**

FIGURE 11

REFRACTION OF GLYCEROL SOLUTION  
USING A HAND-HELD REFRACTOMETER  
(TS METER MODEL 10400A)



Glycerol Concentration W/V  
In 0.9% NaCl + 0.2 gm% Glucose

FIGURE 12.



# RED BLOOD CELL RECOVERY WORKSHEET

56  
REVISED 4/97

UNIT # \_\_\_\_\_ DATE \_\_\_\_\_

## 1. MEASURED VALUES (INPUT: RECOVERY COMPUTER PROGRAM)

			QC: EXPECTED RANGE	TECH
THAWED UNIT:	1. Wt of bag and blood	g	FRZ SHEET VALUE-30G	
	2. Wt of bag	g		
	3. Hematocrit	v%	52-63	
	4. Sup Hb DILUTION=1	mg/dl	<4000	
	5. Hb	g/dl		
	6. pH (or 0 for NO VALUE)	(@22 c)		
	7. Extra K+ (or 0)	mEq/l	8-15	
WASTE:	8. Total Volume	ml		
	9. Sup Hb Spun _____, unspun	mg/dl		
WASHED UNIT:	10. Wt of bag and blood	g		
	11. Wt of bag	g		
	12. Hematocrit	v%		
	13. Sup Hb (spectro)	mg/dl	<200	
	14. Hb	g/dl		
	15. pH (or 0 FOR NO VALUE)	(@22 c)		
	16. Osmo (or 0)	(mOs/kg H2O)	300-400	
	17. Extra K+ (or 0)	mEq/l	0.1-3	
	18. sup hb (chemstrip)	mg/dl	Patch 1-3	

## CALCULATED VALUES: print out from computer (FORMULAS BLOW)

### THAWED UNIT:

_____	g - Weight of blood	= gross wt - bag wt
_____	g/ml - Density	= 1.1 + [(hct - 20)/1000]
_____	ml - Volume of blood	= wt/density
_____	ml - Sup Vol	= vol of blood x (1-hct)
_____	mg/dl - Sup Hb	
_____	mg - Tot Sup Hb/unit	= sup hb (mg/dl) x sup vol / 100
_____	g - Tot Hb/unit	= hb (g/dl) x vol of blood / 100
_____	g - Tot cell Hb/unit	= tot hb (g) - tot sup hb (g)
_____	% - Percent recovery	= tot cell hb / tot hb

### WASTE

_____	g - Total Hb	= (volume / 100) x sup hb (mg/dl)
-------	--------------	-----------------------------------

### WASHED UNIT:

_____	g - Weight of blood	= gross wt - bag wt
_____	g/ml - Density	= 1.0 + [(hct-5)/1000]
_____	ml - Volume of blood	= wt/density
_____	ml - Sup Vol	= vol of blood x (1-hct)
_____	mg - Tot Sup Hb/unit	= sup hb (mg/dl) x sup vol / 100
_____	g - Tot Hb/unit	= hb (g/dl) x vol of blood / 100
_____	g - Tot cell Hb/unit	= tot hb (g) - tot sup hb (g)

_____	% Recovery (Using Waste)	= Tot cell hb tot hb + waste hb
_____	% Recovery (Using Pre-Post)	= Tot cell hb tot hb thawed unit

CALCULATED BY: \_\_\_\_\_

COMMENTS: \_\_\_\_\_

RESULTS ACCEPTED BY: \_\_\_\_\_

UNIT # OR NAME \_\_\_\_\_ DATE \_\_\_\_\_

FILL IN NUMBER OF DAYS STORED:

LIQUID ( 4 C ) STORED RBC \_\_\_\_\_ DAYS OR DEGLYCEROLIZED RBC \_\_\_\_\_ DAYS

## \* MEASURED VALUES

RED CELL INDICES TECH COULTER TECH

\* SPUN HCT: \_\_\_\_\_ % \_\_\_\_\_

\* MANUAL HB: \_\_\_\_\_ g/dl \_\_\_\_\_

\* RBC COUNT: \_\_\_\_\_

## CALCULATED VALUES:

MCV \_\_\_\_\_ fl \_\_\_\_\_

MCH \_\_\_\_\_ pg \_\_\_\_\_

MCHC \_\_\_\_\_ g/dl RBC \_\_\_\_\_

ELECTROLYTES: NA+ K+

ASSAYS: \* SUP \_\_\_\_\_ mEq/L

\* CELLS \_\_\_\_\_ mEq/L

## CALCULATED VALUES:

INTRACELLULAR \_\_\_\_\_ mEq/10<sup>12</sup> RBC

## FORMULA:

$$K+ \text{ INTRACELLULAR (mEq/L)} = (K+ \text{ CELLS} - K+ \text{ SUP} \times 0.03) / 0.97$$
$$K+ \text{ mEq/10}^{12} \text{ RBC} = K+ \text{ INTRACELLULAR} \times \text{MCV}/1000$$

\* SUPT HB: MEASURED \_\_\_\_\_ mg/dl x DILUTION \_\_\_\_\_ = \_\_\_\_\_ mg/dl

OSMOLALITY \_\_\_\_\_ mOsm/kg H<sub>2</sub>O \* pH \_\_\_\_\_

EQUIPMENT USED: SERIAL # SERIAL #

1. SCALE \_\_\_\_\_ 5. pH METER \_\_\_\_\_

2. HCT CENTRIFUGE \_\_\_\_\_ 6. FLAME PHOTOMETER \_\_\_\_\_

3. HEMOGLOBINOMETER \_\_\_\_\_ 7. OSMOMETER \_\_\_\_\_

4. COULTER COUNTER \_\_\_\_\_ 8. SPECTROPHOTOMETER \_\_\_\_\_

## SHIPPING INSTRUCTIONS

### FROZEN RED BLOOD CELLS

Twelve units of frozen red blood cells should be placed into a polystyrene foam shipping container. Approximately 1 inch of crushed dry ice is placed on the bottom of the container. The frozen units are then added to the container as shown in Figure 13. Place an elastic rubber band around one of the top layer units for the attachment of a temperature monitoring device.

One temperature monitoring device should be used to monitor the temperature of the blood products inside the container during shipment.

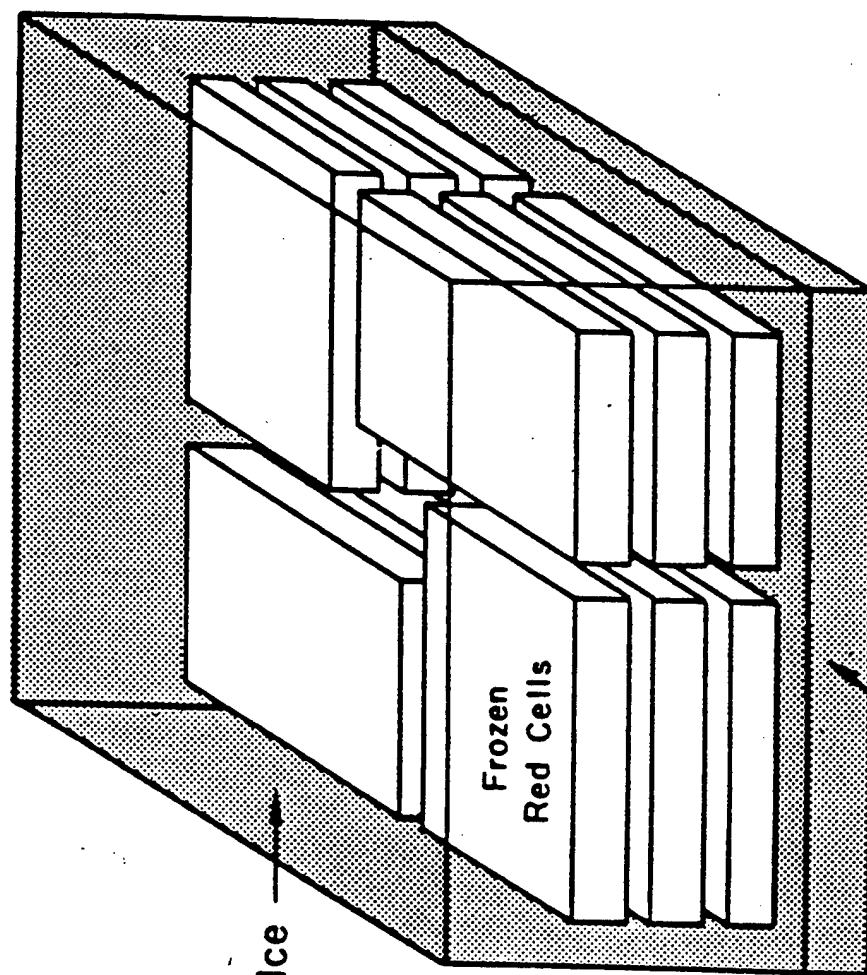
Immediately place the temperature monitoring device under the elastic rubber band which is around one of the top-layer units. This will insure that the temperature monitoring device remains at the top of the product load as the dry ice melts during shipment. Immediately add more dry ice on top of the units so that a total of 40 lbs. has been added to each shipping container.

The shipping container should have a gross weight of at least 55 lbs. prior to shipment to insure that the proper amount of dry ice has been added to the container.

### PREVIOUSLY FROZEN RED BLOOD CELLS

Follow the established procedures for shipment of liquid blood products.

# STANDARD POLYSTYRENE FOAM CONTAINER



Crushed Dry Ice

Frozen  
Red Cells

Box Contains:  
12 units of frozen red cells  
40 lbs of dry ice

Crushed Dry Ice  
(1 inch)

FIGURE 13

## EQUIPMENT LIST

## MINOR EQUIPMENT (LESS THAN \$1,000)

ITEM	PROCEDURE
12-place adaptor for centrifuge (2)	QC
Cylinder, graduated, 4000 ml	Wash
Forceps, Kelly (hemostats)	Coll/Glyc/Wash
Scissors	Coll/Glyc/Wash
Lead weights, 1-2 lbs, no sharp edges (to hold down unit in water bath)	Glyc/Wash
Waterproof felt-tip pens (Sharpie)	Glyc/Wash
Tape dispenser, 1 in.	Coll/Glyc
Integral tube sealer (Sebra 1100)	Coll/Glyc/Wash
Plasma extractor (Fenwal 4R4414)	Coll/Glyc/Wash
Utility water bath (Blue-M MW-1140A)* (Fisher 15-453C)	Glyc/Wash
Pump, circulating (Thomas Scientific 7887-F10), chain clamp (Fisher 05-745), clamp holder (Fisher 05-754) and Stand (Fisher 14-668)	Glyc/Wash
Impulse sealer (Stericon 210X)	Glyc
Thermometer, -100 C - +50 C	Glyc
Thermometer, 0 C - +100 C	Glyc/Wash
Shaker magnets (Haemonetics 9437) (20 needed)	Glyc
Rubber balance discs (Sorval 00335)	Coll/Glyc/Wash
Tubing stripper/hand sealer (Fenwal 4R4417)	Coll/Glyc/Wash
Eberbach Shaker (Eberbach 2900) or Eberbach 6010 modified with Eberbach 2910	Glyc
Balance, top loading (Mettler PE-6000)	Coll/Glyc/Wash
Adjustable volume pipettors, Gilson (200 microliter and 1000 microliter)	QC
Hand digital tachometer (Shimpo DT-207)	QC

Hand held refractometer  
(TS Meter Model 10400A)

QC

Infrared scanner (Exergen D-501F)

Glyc/Thaw

\*Forma water baths or similar baths with coils not submerged in water are not recommended.

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**MAJOR EQUIPMENT**

ITEM	PROCEDURE
22 C refrigerated centrifuge (Dupont RC-3B or Beckman J6B), 4 head rotor	Coll/Glyc/Wash
-80 C mechanical freezer (chest-type, min. 1 HP compressor (Harris or So-Low)	Freeze/Storage
Blood processor (Haemonetics 115)	Wash
4 C blood bank refrigerator	Storage
Flame photometer (IL 943)	QC
Osmometer (Fiske 2400)	QC
Refractometer, Abbe (AO-10480; Fisher 13-975-100)	QC
Constant temp. water bath (Haake A80; Fisher 13-875-112A)	QC
Spectrophotometer (Spectronic 21/MV, Fisher 14-385-360); Cuvettes: Fisher 14-377-280)	QC
37 C incubator (Fisher IL-990)	QC
Sterile docking device (Terumo)	Glyc/Wash
Microfuge (IEC MB)	QC
Table top centrifuge (Sorvall RT6000B)	QC

## VENDORS

## ITEM

Beckman Instruments  
607 North Avenue  
Wakefield, MA  
617-245-6800

Centrifuge

Cambridge Instruments Co.  
MISCO Products Division  
3401 Virginia Road  
Cleveland, OH 44122  
216-831-1000

Hand Held  
Refractometer  
(TS Meter)

Medsep Corporation  
1630 Industrial Park Street  
Covina, CA 91722  
800-288-8379

800 ml primary  
collection bag

Cytosol Labs, Inc  
55 Messina Drive  
Braintree, MA 02184  
617-848-9387

Glycerol

E. I. duPont Company  
Sorvall Products Division  
McKean Building, Concord Plaza  
Wilmington, DE 19898  
302-774-1000

Centrifuge

Engineering & Research  
Associates, Inc.  
500 North Tucson Blvd.  
Tucson, AZ 85716  
602-881-6555

Sebra sealer

Exergen Corporation  
51 Water Street  
Watertown, MA 02172  
617-527-6660

Infrared microscanner

Fenwal Laboratories  
1425 Lake Cooke Road  
Deerfield, IL 60015  
708-940-5818

Glycerol, 12%  
sodium chloride,  
0.9% sodium chloride-  
0.2% glucose, 800 ml  
primary collection  
bag

Fiske Associates  
2 Technology Way  
Norwood, MA 02062  
617-320-5656

Osmometer

Haemonetics Corp.  
400 Wood Road  
Braintree, MA 02184  
617-848-7100

Blood Processor  
115, cell wash sets,  
recovery bags

Harris Manufacturing Co., Inc.  
275 Aiken Road, Route 1  
Asheville, NC 28804  
704-658-2711

-80 C freezer

Kapak Corp  
5305 Parkdale Drive  
Minneapolis, MN 55416  
612-541-0730

Heat sealable  
polyester  
plastic bags

Shimpo  
3510 Devon Avenue  
Lincolnwood, IL 60659  
312-679-6765

Tachometer

Sigma Diagnostics  
P.O. Box 14508  
St. Louis, MO 63178  
800-325-3010

Total Hemoglobin  
Standard Kit

So-Low Environmental Equip. Co.  
10310 Spartan Drive  
Cincinnati, OH 45215  
513-772-9410

-80 C Freezer

Stericon, Inc.  
2315 Gardner Road  
Broadview, IL 60153  
708-865-8790

Impulse sealer

Stone Container Corp.  
1900C Industrial Boulevard  
P.O. Box 847  
Temple, TX 76502  
817-778-4837

Frozen blood box,  
printed

Terumo Corporation  
2100 Cottontail Lane  
Somerset, NY 08873  
908-302-4900

Sterile docking wafers,  
sterile docking device

Thomas Scientific  
99 High Hill Road  
Swedesboro, NJ 08085  
800-345-2103

Circulating  
water pump